Ref #	Hits	Search Query	DBs	Default Operator	Plurals	Time Stamp
L1	2	humanized and gelonin.ab.	US-PGPUB; USPAT; DERWENT	ADJ	ON	2005/08/16 10:58
L2	277	humanized and enzyme?.ab.	US-PGPUB; USPAT; DERWENT	ADJ	ON	2005/08/16 10:58
L3	17	humanized.ab. and enzyme?.ab.	US-PGPUB; USPAT; DERWENT	ADJ	ON	2005/08/16 11:01
L4	35	humanized.ab. and toxin.ab.	US-PGPUB; USPAT; DERWENT	ADJ	ON	2005/08/16 11:09
L5	0	humanized ricin	US-PGPUB; USPAT; DERWENT	ADJ	ON	2005/08/16 11:09
L6	0	humanized ?toxin?	US-PGPUB; USPAT; DERWENT	ADJ	ON	2005/08/16 11:09
L7	0	humanised ?toxin?	US-PGPUB; USPAT; DERWENT	ADJ	ON	2005/08/16 11:10
L8	3	epitope replacement	US-PGPUB; USPAT; DERWENT	ADJ	ON	2005/08/16 11:10
L9	0	epitope swap	US-PGPUB; USPAT; DERWENT	ADJ	ON	2005/08/16 11:10

10/074596
File 5:Biosis Previews(R) 1969-2005/Aug W1
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Set	Items	Description
S1	2	HUMANIZED(5W)TOXIN?
S2	4	HUMANIZED(5W)ENZYME?
S3	957	EPITOP? AND REPLACE?
S4	496	S3 AND HUMAN?
S5	0	SSSSSS
S6	0	EPITOPE SWAP
S7	91	ENZYME AND EPITOPE AND REPLACE?
S8	44	S7 AND HUMAN?
S9	21	TOXIN AND EPITOPE AND REPLACE?

? t s1/7/1-2

1/7/1
DIALOG(R)File 5:Biosis Previews(R)

0009660419 BIOSIS NO.: 199598128252
Future of Monoclonal Antibodies in Solid Organ Transplantation
AUTHOR: Cosimi A Benedict
AUTHOR ADDRESS: Dep. Surg., Massachusetts Gen. Hosp., Boston, MA 02114, USA **USA
JOURNAL: Digestive Diseases and Sciences 40 (1): p65-72 1995 1995
ISSN: 0163-2116
DOCUMENT TYPE: Article; Literature Review
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The ultimate objective of immunosuppressive therapy is to block transplant recipient reactivity to allograft incompatibilities while sparing other responses. Increased clarification of rejection mechanisms has made possible the precise suppression of specific elements of the immune response using murine anti-human monoclonal antibodies. In addition, recombinant DNA technology has made available novel agents including "%%%humanized%%%," bispecific, or %%%toxin%%%-conjugated molecules, which avoid some of the limitations of murine reagents. Using such agents, donor-specific tolerance has been induced in experimental models after a limited course of therapy directed against selected effector cell surface-associated molecules such as CD4, CD25, and CD54. It remains to be determined how such observations can be successfully transferred to the human situation. It seems likely, however, that as new molecular agents are developed, increasingly effective suppression of specific cellular targets will become an essential element of clinical protocols. Such agents may provide long-term immunosuppression with limited periods of immunosuppressive agent administration.

1/7/2
DIALOG(R)File 5:Biosis Previews(R)

0009648845 BIOSIS NO.: 199598116678
Efficient generation of a reshaped human mAb specific for the alpha toxin of Clostridium perfringens
AUTHOR: Tempest Philip R; White Patricia; Williamson E Diane; Titball Richard W; Kelly David C; Kemp Graham J L; Gray Peter M D; Forster Simon J; Carr Frank J; Harris William J (Reprint)
AUTHOR ADDRESS: Scotgen Biopharm. Inc., Kettock Lodge, Campus 2, Aberdeen Sci. Technol. Park, Bridge of Don, Aberdeen AB22 9GU, UK**UK
JOURNAL: Protein Engineering 7 (12): p1501-1507 1994 1994
ISSN: 0269-2139
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: We have used the technique of antibody reshaping to produce a %%%humanized%%% antibody specific for the cc %%%toxin%%% of Clostridium perfringens. The starting antibody was from a mouse hybridoma from which variable (V) region nucleotide sequences were determined. The complementarity-determining regions (CDRs) from these V regions were then inserted into human heavy and light chain V region genes with human constant region gene fragments subsequently added. The insertion of CDRs alone into human frameworks did not produce a functional reshaped antibody and modifications to the V region framework were required. With minor framework modifications, the affinity of the original murine mAb was restored and even exceeded. Where affinity was increased, an altered binding profile to overlapping peptides was observed. Computer modelling of the reshaped heavy chain V regions suggested that amino acids adjacent to CDRs can either contribute to, or distort, CDR loop conformation and must be adjusted to achieve high binding affinity.

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2/7/1

DIALOG(R)File 5:Biosis Previews(R)

0015208884 BIOSIS NO.: 200500115062

Two novel mutant human adenylosuccinate Lyases (ASLs) associated with autism and characterization of the equivalent mutant Bacillus subtilis ASL

AUTHOR: Sivendran Sharmila; Patterson David; Spiegel Erin; McGown Ivan; Cowley David; Colman Roberta F (Reprint)

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JOURNAL: Journal of Biological Chemistry 279 (51): p53789-53797 December 17, 2004 2004

MEDIUM: print

ISSN: 0021-9258

DOCUMENT TYPE: Article

RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: An Australian patient with autism was found to be heterozygous for two mutations in the gene encoding adenylosuccinate lyase (ASL), resulting in the protein mutations E80D and D87E. The patient's mother carried only the E80D mutation. The equivalent positions are 62 and 69 in Bacillus subtilis ASL. Although both human and B. subtilis enzymes normally have Asp at position 87 (or 69), the B. subtilis ASL has Ile and Asp at 62 and 65, respectively, whereas human ASL has Glu and Arg at the equivalent positions. We have constructed, expressed, and purified the double mutant I62E/D65R as a "%%%humanized%%%" normal B. subtilis %%%enzyme%%% to compare with enzymes with a single mutation at position 62 (I62D/D65R), at position 69 (I62E/D65R/D69E), or at both positions (I62D/D65R/D69E). Vmax for conversion of adenylosuccinate to AMP and fumarate is 0.57 mumol/min/mg for I62E/D65R, 0.064 mumol/min/mg for I62D/D65R, 0.27 mumol/min/mg for I62E/D65R/D69E, and 0.069 mumol/min/mg for I62D/D65R/D69E. The Km for adenylosuccinate is elevated in the X62D mutants, and I62D/D65R is the least stable of these ASLs at 37 degreeC. The CD spectra of mutant and wild type enzymes are similar; thus, there are no appreciable structural changes. Clearly the Asp62 causes the most drastic effect on ASL function, whereas the Glu69 mutation produces only modest change. These results emphasize the importance of expanding tests for ASL deficiency to individuals with developmental delay of any severity, including individuals with autistic spectrum disorder. This study further demonstrates the usefulness of the B. subtilis ASL as a model to mimic the defective enzyme in ASL deficiency.

2/7/2

DIALOG(R)File 5:Biosis Previews(R)

0013218358 BIOSIS NO.: 200100390197

Thiazolidinediones but not metformin directly inhibit the steroidogenic

enzymes P450c17 and 3beta-hydroxysteroid dehydrogenase
AUTHOR: Arlt Wiebke; Auchus Richard J; Miller Walter L (Reprint)
AUTHOR ADDRESS: Dept. of Pediatrics, University of California, Bldg. MR-IV, Rm. 209, San Francisco, CA, 94143-0978, USA**USA
JOURNAL: Journal of Biological Chemistry 276 (20): p16767-16771 May 18, 2001 2001
MEDIUM: print
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Androgen biosynthesis requires 3beta-hydroxysteroid dehydrogenase type II (3betaHSDII) and the 17alpha-hydroxylase and 17,20-lyase activities of cytochrome P450c17. Thiazolidinedione and biguanide drugs, which are used to increase insulin sensitivity in type 2 diabetes, lower serum androgen concentrations in women with polycystic ovary syndrome. However, it is unclear whether this is secondary to increased insulin sensitivity or to direct effects on steroidogenesis. To investigate potential actions of these drugs on P450c17 and 3betaHSDII, we used " %%%humanized%%% yeast" that express these steroidogenic %%%enzymes%%% in microsomal environments. The biguanide metformin had no effect on either enzyme, whereas the thiazolidinedione troglitazone inhibited 3betaHSDII (KI = 25.4 +- 5.1 muM) and both activities of P450c17 (KI for 17alpha-hydroxylase, 8.4 +- 0.6 muM; KI for 17,20-lyase, 5.3 +- 0.7 muM). The action of troglitazone on P450c17 was competitive, but it was mainly a noncompetitive inhibitor of 3betaHSDII. The thiazolidinediones rosiglitazone and pioglitazone exerted direct but weaker inhibitory effects on both P450c17 and 3betaHSDII. These differential effects of the thiazolidinediones do not correlate with their effects on insulin sensitivity, suggesting that distinct regions of the thiazolidinedione molecule mediate these two actions. Thus, thiazolidinediones inhibit two key enzymes in human androgen synthesis contributing to their androgen-lowering effects, whereas metformin affects androgen synthesis indirectly, probably by lowering circulating insulin concentrations.

2/7/3
DIALOG(R)File 5:Biosis Previews(R)

0012592276 BIOSIS NO.: 200000310589
Design and synthesis of cyclopenta(g)quinazoline-based antifolates as inhibitors of thymidylate synthase and potential antitumor agents
AUTHOR: Bavetsias Vassilios (Reprint); Marriott Jonathan H; Melin Camille; Kimbell Rosemary; Matusiak Zbigniew S; Boyle F Thomas; Jackman Ann L
AUTHOR ADDRESS: CRC Centre for Cancer Therapeutics Cancer Research Campaign Laboratories, The Institute of Cancer Research, 15 Cotswold Road, Sutton, Surrey, SM2 5NG, UK**UK
JOURNAL: Journal of Medicinal Chemistry 43 (10): p1910-1926 May 18, 2000 2000
MEDIUM: print
ISSN: 0022-2623
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Following the development of raltitrexed, the synthesis of nonpolyglutamatable inhibitors of TS that do not use the reduced folate carrier (RFC) for cellular entry should provide compounds which overcome mechanisms of resistance to folate-based inhibitors of TS that are associated with decreased/altered folylpolyglutamate synthetase (FPGS) expression and/or an impaired RFC. Examination of a computer graphics model of the %%%humanized%%% Escherichia coli TS %%%enzyme%%% with quinazoline inhibitors of TS, such as 1 bound in the active site of the enzyme, suggested that conformational restriction introduced by bridging the C9 with C7 to form a pentacycle may be beneficial for binding to TS. That led to the synthesis of a series of potent cyclopenta(g)quinazoline-based inhibitors of the enzyme in which the glutamyl residue associated with classical antifolates was replaced with a variety of glutamate-derived ligands; the most potent inhibitor being

the L-Glu-gamma-D-GluTalpha derivative 7j. In the mouse L1210:1565 cell line (mutant RFC), the majority of these compounds had activity equal or only slightly greater compared with the parental L1210 cell line, indicating a reduced dependence on the RFC for cellular uptake in the L1210 cell line.

2/7/4
DIALOG(R)File 5:Biosis Previews(R)
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0010262949 BIOSIS NO.: 199698730782
Toward antibody-directed "abzyme" prodrug therapy, ADAPT: Carbamate prodrug activation by a catalytic antibody and its in vitro application to human tumor cell killing
AUTHOR: Wentworth Paul; Datta Anita; Blakey David; Boyle Tom; Partridge Lynda J; Blackburn G Michael (Reprint)
AUTHOR ADDRESS: Dep. Mol. Biol., Krebs Inst. Biomol. Res., Univ. Sheffield, Sheffield S3 7HF, UK**UK
JOURNAL: Proceedings of the National Academy of Sciences of the United States of America 93 (2): p799-803 1996 1996
ISSN: 0027-8424
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Antibody-directed enzyme prodrug therapy, ADEPT, is a recent approach to targeted cancer chemotherapy intended to diminish the nonspecific toxicity associated with many commonly used chemotherapeutic agents. Most ADEPT systems incorporate a bacterial enzyme, and thus their potential is reduced because of the immunogenicity of that component of the conjugate. This limitation can be circumvented by the use of a catalytic antibody, which can be "%%%humanized%%%," in place of the bacterial %%%enzyme%%% catalyst. We have explored the scope of such antibody-directed "abzyme" prodrug therapy, ADAPT, to evaluate the potential for a repeatable targeted cancer chemotherapy. We report the production of a catalytic antibody that can hydrolyze the carbamate prodrug
4-(N,N-bis(2-chloroethyl))aminophenyl-N-((1S)-(1,3-dicarboxy)propyl)carbamate (1) to generate the corresponding cytotoxic nitrogen mustard (K-m = 201 mu-M, k-cat 1.88 min-1). In vitro studies with this abzyme, EA11-D7, and prodrug 1 lead to a marked reduction in viability of cultured human colonic carcinoma (LoVo) cells relative to appropriate controls. In addition, we have found a good correlation between antibody catalysis as determined by this cytotoxicity assay in vitro and competitive binding studies of candidate abzymes to the truncated transition-state analogue ethyl 4-nitrophenylmethylphosphonate. This cell-kill assay heralds a general approach to direct and rapid screening of antibody libraries for catalysts.
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? t s4/7/100-125

4/7/100
DIALOG(R)File 5:Biosis Previews(R)
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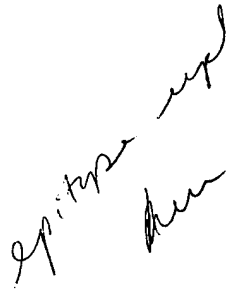
0013687076 BIOSIS NO.: 200200280587
A Y2 receptor mimetic aptamer directed against neuropeptide Y
AUTHOR: Proske Daniela; Hoefliger Martin; Soell Richard M; Beck-Sickinger Annette G (Reprint); Famulok Michael (Reprint)
AUTHOR ADDRESS: Kekule Institut fuer Organische Chemie und Biochemie, Universitaet Bonn, Gerhard Domagk Strasse 1, 53121, Bonn, Germany** Germany
JOURNAL: Journal of Biological Chemistry 277 (13): p11416-11422 March 29, 2002 2002
MEDIUM: print
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Neuropeptide Y (NPY) is a 36-amino acid neuropeptide that exerts its activity by at least five different receptor subtypes that belong to the family of G-protein-coupled receptors. We isolated an aptamer directed against NPY from a nuclease-resistant RNA library. Mapping experiments with N-terminally, C-terminally, and centrally truncated analogues of NPY revealed that the aptamer recognizes the C terminus of NPY. Individual %%%replacement%%% of the four arginine residues at positions 19, 25, 33, and 35 by L-alanine showed that arginine 33 is essential for binding. The aptamer does not recognize pancreatic polypeptide, a highly homologous Y4 receptor-specific peptide of the gut. Furthermore, the affinity of the aptamer to the Y5 receptor-selective agonist (Ala31,Aib32)NPY and the Y1/Y5 receptor-binding peptide (Leu31,Pro34)NPY was considerably reduced, whereas Y2 receptor-specific NPY mutants were bound well by the aptamer. Accordingly, the NPY %%%epitope%%% was recognized by the Y2 receptor, and the aptamer was highly similar. This Y2 receptor mimicking effect was further confirmed by competition binding studies. Whereas the aptamer competed with the Y2 receptor for binding of (3H)NPY with high affinity, a low affinity displacement of (3H)NPY was observed at the Y1 and the Y5 receptors. Consequently, competition at the Y2 receptor occurred with a considerably lower Ki value compared with the Y1 and Y5 receptors. These results indicate that the aptamer mimics the binding of NPY to the Y2 receptor more closely than to the Y1 and Y5 receptors.



0013674354 BIOSIS NO.: 200200267865
New molecular targets of breast cancer therapy
AUTHOR: Sauer Georg (Reprint); Deissler Helmut; Kurzeder Christian; Kreienberg Rolf
AUTHOR ADDRESS: Department of Gynecology and Obstetrics, University of Ulm Medical School, Prittwitzstrasse 43, 89075, Ulm, Germany**Germany
JOURNAL: Strahlentherapie und Onkologie 178 (3): p123-133 Marz, 2002 2002
MEDIUM: print
ISSN: 0179-7158
DOCUMENT TYPE: Article; Literature Review
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Background: The development of new chemotherapeutic agents and concepts of radiation therapy, administered as primary, adjuvant and palliative therapy, has led to new perspectives in breast cancer therapy. Apart from conventional chemotherapy, recently developed novel agents interfere with molecular mechanisms that are altered in cancer cells. Those targets are not necessarily breast cancer-specific. In this review we will focus on novel agents with potential or already proved benefit to breast cancer patients. Promising strategies include inhibition of growth factor receptors, blocking of tumor angiogenesis and signal transduction pathways, modulation of apoptosis, cancer vaccination, and inhibition of invasion and metastasis. Methods: Reports of relevant studies obtained from a search of MEDLINE and studies referenced in those reports were reviewed. Results: Apart from trastuzumab, other further developed compounds show promising results in clinical studies as a second generation of growth factor inhibitors. Different approaches in anti-angiogenetic therapy are under preclinical and clinical phase-II trials. Pro-apoptotic agents show synergistic effects with docetaxel in a clinical phase-I trial. Other compounds that target HSP 90, histone deacetylase and HMG-CoA reductase target atypical apoptotic pathways being lethal to tumor cells only but not to normal tissue, suggesting a tumor-specific way of action. MMP inhibitors have been demonstrating promising results in patients with refractory malignant pleural effusion in a phase-I trial. Several tyrosine kinase inhibitors currently under clinical investigation preliminarily show hopeful results in patients with advanced breast cancer. Furthermore, recent progress in defining the immunogenic %%%epitopes%%% of tumor antigens has rejuvenated the interest in cancer vaccines. Conclusion: Typical dose escalation studies leading to the highest clinically still tolerated dose do not appear to be equally appropriate for the estimation of efficiency of those compounds

as for conventional cytotoxic regimes. Rather, escalation up to an amount of therapeutic agent that is sufficient for maximum target inhibition should be promoted, where classical measures of cytoreduction such as complete or partial remission are %%%replaced%%% both by time to progression and treatment failure as an appropriate measure of the efficacy of an agent.

4/7/102
DIALOG(R)File 5:Biosis Previews(R)

0013668714 BIOSIS NO.: 200200262225
Two classes of germline genes both derived from the VH1 family direct the formation of %%%human%%% antibodies that recognize distinct antigenic sites in the C2 domain of factor VIII
AUTHOR: Van Den Brink Edward N; Bril Wendy S; Turenhout Ellen A M; Zuurveld Marleen; Bovenschen Niels; Peters Marjolein; Yee Thynn Thynn; Mertens Koen; Lewis Deborah A; Ortel Thomas L; Lollar Pete; Scandella Dorothea; Voorberg Jan (Reprint)
AUTHOR ADDRESS: Dept of Plasma Proteins, CLB, Plesmanlaan 125, 1066 CX, Amsterdam, Netherlands**Netherlands
JOURNAL: Blood 99 (8): p2828-2814 April 15, 2002 2002
MEDIUM: print
ISSN: 0006-4971
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Most plasmas from patients with inhibitors contain antibodies that are reactive with the C2 domain of factor VIII. Previously, we have shown that the variable heavy chain (VH) regions of antibodies to the C2 domain are encoded by the closely related germline gene segments DP-10, DP-14, and DP-88, which all belong to the VH1 gene family. Here, we report on the isolation and characterization of additional anti-C2 antibodies that are derived from VH gene segments DP-88 and DP-5. Competition experiments using murine monoclonal antibodies CLB-CAg 117 and ESH4 demonstrated that antibodies derived from DP-5 and DP-88 bound to different sites within the C2 domain. %%%Epitope%%% mapping studies using a series of factor VIII/factor V hybrids revealed that residues 2223 to 2332 of factor VIII are required for binding of the DP-10-, DP-14-, and DP-88-encoded antibodies. In contrast, binding of the DP-5-encoded antibodies required residues in both the amino- and carboxy-terminus of the C2 domain. Inspection of the reactivity of the antibodies with a series of %%%human%%%/porcine hybrids yielded similar data. Binding of antibodies derived from germline gene segments DP-10, DP-14, and DP-88 was unaffected by %%%replacement%%% of residues 2181 to 2243 of %%%human%%% factor VIII for the porcine sequence, whereas binding of the DP-5-encoded antibodies was abrogated by this %%%replacement%%%. Together these data indicate that antibodies assembled from VH gene segments DP-5 and the closely related germline gene segments DP-10, DP-14, and DP-88 target 2 distinct antigenic sites in the C2 domain of factor VIII.

4/7/103
DIALOG(R)File 5:Biosis Previews(R)

0013668056 BIOSIS NO.: 200200261567
Reduction in the immunogenicity of %%%human%%% FVIII in hemophilia A mice by site-directed mutagenesis of the A2 %%%epitope%%%
AUTHOR: Parker Ernest T (Reprint); Craddock Heather N (Reprint); Barrow Rachel T (Reprint); Healey John F (Reprint); Lollar Pete (Reprint)
AUTHOR ADDRESS: Winship Cancer Institute, Emory University, Atlanta, GA, USA**USA
JOURNAL: Blood 98 (11 Part 1): p826a November 16, 2001 2001
MEDIUM: print
CONFERENCE/MEETING: 43rd Annual Meeting of the American Society of Hematology, Part 1 Orlando, Florida, USA December 07-11, 2001; 20011207
SPONSOR: American Society of Hematology

ISSN: 0006-4971
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Intravenous %%%human%%% factor VIII (fVIII) %%%replacement%%% therapy is the mainstay of treatment of patients with hemophilia A. Approximately 25% percent of patients develop inhibitory antibodies ("inhibitors") to fVIII, which severely complicates clinical management. Immunodominant B cell %%%epitopes%%% that bind patient antibodies have been identified in the A2, ap-A3, and C2 domains of fVIII. The A2 %%%epitope%%% apparently is confined to a segment consisting of residues R484-I508. Within this region, several antigenically important residues have been identified, including R484, Y487, S488, R489, P492, V495, F501, and I508 (Lubin, I.M. et al. J. Biol. Chem. 272:30191-30195, 1997). Hemophilia A mice created by targeted disruption of either exon 16 or exon 17 of the fVIII gene develop high titer inhibitory antibodies when infused intravenously with %%%human%%% fVIII according to a dosage schedule that simulates treatment in man (Qian, J., et al. Thromb. Haemost. 81:240-244, 1999). We tested the hypothesis that the immunogenicity of fVIII in this model can be reduced by mutagenesis of antigenic A2 residues. C57BL/6J fVIII exon 16-disrupted mice were randomized to two groups receiving either B domain-deleted "wild-type" fVIII, HSQ, (n=24) or a R484A/R489A/P492A mutant in the HSQ background (n=23). Mice received six doses of 10 mug/kg intravenously every two weeks, followed two weeks later by a final dose of 25 mug/kg. Two weeks after the last dose, blood was obtained by cardiac puncture and the inhibitor titer was measured using the Bethesda assay. The Bethesda titer of the HSQ group was significantly higher than the R484A/R489A/P492A mutant group, 669+-318 versus 318+-310 (mean+-sample standard deviation, p=0.01, t-test). Samples from the HSQ group also were tested for inhibitory activity against a hybrid %%%human%%%/porcine fVIII molecule, HP9, which contains substitution of the porcine 484-508 A2 %%%epitope%%% segment for the corresponding %%%human%%% sequence. The Bethesda titer toward HSQ was 322+-342 units higher than toward HP9 (mean difference+-sample standard deviation, p=0.0002, paired t-test), indicating that the murine immune system recognizes the %%%human%%% fVIII inhibitory A2 %%%epitope%%%. We conclude that the immunogenicity of %%%human%%% fVIII can be reduced by mutagenesis of B cell %%%epitopes%%%. This approach may lead to improved factor VIII products in the treatment of hemophilia A.

0013655273 BIOSIS NO.: 200200248784
Differential expression of %%%human%%% chorionic gonadotropin (hCG) glycosylation isoforms in failing and continuing pregnancies: Preliminary characterization of the hyperglycosylated hCG %%%epitope%%%
AUTHOR: Kovalevskaya G; Birken S; Kakuma T; Ozaki N; Sauer M; Lindheim S; Cohen M; Kelly A; Schlatterer J; O'Connor J F (Reprint)
AUTHOR ADDRESS: Irving Center for Clinical Research, Columbia University College of Physicians and Surgeons, 630 W 168th St, PH10-305, New York, NY, 10032, USA**USA
JOURNAL: Journal of Endocrinology 172 (3): p497-506 March, 2002 2002
MEDIUM: print
ISSN: 0022-0795
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: %%%Human%%% chorionic gonadotropin (hCG) glycoforms change as pregnancy progresses. We have developed an antibody (B152) which can measure a hyperglycosylated early pregnancy isoform of hCG. This putative hyperglycosylated form of hCG arises very early in pregnancies and is rapidly %%%replaced%%% by an isoform that predominates for the remainder of the pregnancy. The profiles of these hCG glycoforms are measured as a ratio of values of two immunometric assays. The profiles of these ratios differ between pregnancies which persist and those which will experience

early failure. In this report, daily urine hCG isoform ratios from donor eggs (no exogenous hCG pretreatment), in vitro fertilization pregnancies were profiled and analyzed from the first day following embryo transfer (ET). Significant differences were found between continuing pregnancy and pregnancy loss throughout days 5-20 post-ET. When hCG isoform ratios were analyzed from the first day of detectable hCG, pregnancy loss could be predicted in the case of a single fetus both during the 5- to 10-day time segment (P=0.018) and the 10- to 15-day time segment (P=0.045). When single and multiple fetus pregnancies were analyzed together significance was approached in the 10- to 15-day time period (P=0.058). In a second population of pregnant women who conceived naturally, in whom urine samples were collected at approximately weekly intervals to either term birth or clinical spontaneous abortion, the ratio could discriminate between miscarriages and normal term pregnancies (P=0.043). In later pregnancy, the ratio of hCG isoforms declined more rapidly in miscarriages than in term pregnancy. Antibody B152 was produced using a choriocarcinoma-derived hCG (C5), which was hyperglycosylated at both N- and O-linked sites and was 100% nicked at position beta47-48. Western blot analyses supported the assay results showing that early pregnancy urine does not contain nicked C5-like hCG. Also, the early pregnancy hCG appeared to be the same size as later pregnancy hCG as judged by SDS gel electrophoresis. A series of Western blot analyses and immunoassays conducted with the samples either non-reduced or reduced showed that B152 is directed to a linear %%%epitope%%% located in the COOH-terminal peptide region of the beta subunit. This indicated that only the O-glycan groups and not the N-linked glycans are part of the antibody %%%epitope%%%.

4/7/105
DIALOG(R)File 5:Biosis Previews(R)

0013623594 BIOSIS NO.: 200200217105
Structure of a phage display-derived variant of %%%human%%% growth hormone complexed to two copies of the extracellular domain of its receptor: Evidence for strong structural coupling between receptor binding sites
AUTHOR: Schiffer Celia; Ultsch Mark; Walsh Scott; Somers William; de Vos Abraham M; Kossiakoff Anthony (Reprint)
AUTHOR ADDRESS: Department of Protein Engineering, Genentech Inc., 1 DNA Way, South San Francisco, CA, 94080, USA**USA
JOURNAL: Journal of Molecular Biology 316 (2): p277-289 15 February, 2002 2002
MEDIUM: print
ISSN: 0022-2836
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The structure of the ternary complex between the phage display-optimized, high-affinity Site 1 variant of %%%human%%% growth hormone (hGH) and two copies of the extracellular domain (ECD) of the hGH receptor (hGHR) has been determined at 2.6 ANG resolution. There are widespread and significant structural differences compared to the wild-type ternary hGH hGHR complex. The hGH variant (hGHv) contains 15 Site 1 mutations and binds > 102 tighter to the hGHR ECD (hGHR1) at Site 1. It is biologically active and specific to hGHR. The hGHv Site 1 interface is somewhat smaller and 20% more hydrophobic compared to the wild-type (wt) counterpart. Of the ten hormone-receptor H-bonds in the site, only one is the same as in the wt complex. Additionally, several regions of hGHv structure move up to 9 ANG in forming the interface. The contacts between the C-terminal domains of two receptor ECDs (hGHR1-hGHR2) are conserved; however, the large changes in Site 1 appear to cause global changes in the domains of hGHR1 that affect the hGHv-hGHR2 interface indirectly. This coupling is manifested by large changes in the conformation of groups participating in the Site 2 interaction and results in a structure for the site that is reorganized extensively. The hGHv-hGHR2 interface contains seven H-bonds, only one of which is found in the wt complex. Several groups on hGHv and hGHR2 undergo conformational changes of up to 8 ANG. Asp116 of hGHv plays a central role in the reorganization of Site 2 by forming two new H-bonds

to the side-chains of Trp104R2 and Trp169R2, which are the key binding determinants of the receptor. The fact that a different binding solution is possible for Site 2, where there were no mutations or binding selection pressures, indicates that the structural elements found in these molecules possess an inherent functional plasticity that enables them to bind to a wide variety of binding surfaces.

4/7/106
DIALOG(R)File 5:Biosis Previews(R)

0013606131 BIOSIS NO.: 200200199642
HPA-1a phenotype-genotype discrepancy reveals a naturally occurring Arg93Gln substitution in the platelet beta3 integrin that disrupts the HPA-1a %%%epitope%%%
AUTHOR: Watkins Nicholas A; Schaffner-Reckinger Elisabeth; Allen David L; Howkins Graham J; Brons Nicolaas H C; Smith Graham A; Metcalfe Paul; Murphy Michael F; Kieffer Nelly; Ouwehand Willem H (Reprint)
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JOURNAL: Blood 99 (5): p1833-1839 March 1, 2002 2002
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A single nucleotide polymorphism (SNP) at position 196 in the beta3 integrin causes a Leu33Pro substitution in the mature protein. Alloimmunization against the beta3Leu33 form (%%%human%%% platelet antigen (HPA)-1a, PIA1, Zwa) in patients who are beta3Pro33 homozygous (HPA-1b1b, PIA2A2, Zwbb) causes neonatal alloimmune thrombocytopenia, posttransfusion purpura, or refractoriness to platelet transfusion. Studies with recombinant proteins have demonstrated that amino acids 1 to 66 and 288 to 490 of the beta3 integrin contribute to HPA-1a %%%epitope%%% formation. In determining the HPA-1a status of more than 6000 donors, we identified a donor with an HPA-1aweak phenotype and an HPA-1a1b genotype. The platelets from this donor had normal levels of surface alphaIIbbeta3 but reacted only weakly with monoclonal and polyclonal anti-HPA-1a by whole blood enzyme-linked immunosorbent assay (ELISA), flow cytometry, and sandwich ELISA. We reasoned that an alteration in the primary nucleotide sequence of the beta3Leu33 allele of this donor was disrupting the HPA-1a %%%epitope%%%. In agreement with this hypothesis, sequencing platelet RNA-derived alphaIIb and beta3 cDNA identified a novel G/A SNP at position 376 of the beta3 integrin that encodes for an Arg93Gln %%%replacement%%% in the beta3Leu33 allele. Coexpression of the beta3Leu33Gln93 encoding cDNA in Chinese hamster ovary cells with %%%human%%% alphaIIb cDNA showed that the surface-expressed alphaIIbbeta3 reacted normally with beta3 integrin-specific monoclonal antibodies but only weakly with monoclonal anti-HPA-1a. Our results show that an Arg93Gln mutation in the beta3Leu33 encoding allele disrupts the HPA-1a %%%epitope%%%, suggesting that Arg93 contributes to the formation of the HPA-1a B-cell %%%epitope%%%.

4/7/107
DIALOG(R)File 5:Biosis Previews(R)

0013574142 BIOSIS NO.: 200200167653
Mapping of natural anti-factor VIII antibodies in plasma pools from healthy donors: Use of rationally designed synthetic peptides
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JOURNAL: Biologicals 29 (3-4): p229-232 Sept.-Dec., 2001 2001
MEDIUM: print
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RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: Inhibitor antibodies of blood coagulation factor VIII (FVIII) impair FVIII %%%replacement%%% therapy, constituting a serious complication in haemophilic patients. anti-FVIII antibodies may also develop in a variety of disease-associated autoimmunity. Mapping of %%%human%%% FVIII inhibitors in haemophilia A or autoantibody origin have delineated three major clusters of B-cell inhibitory %%%epitopes%%% (domain A2, A3 and C2). Inhibitory and non-inhibitory FVIII antibodies have also been described in plasma of healthy donors and pools of immunoglobulins. The purpose of this study was to use synthetic FVIII-peptides to more closely define regions of the molecule targeted by natural anti-FVIII antibodies. Predictive algorithms were used for defining the positions of potential continuous %%%epitopes%%%. To investigate the presence of peptide-reactive antibodies in normal plasma pools of healthy donors, a plasma fraction (Cohn fraction II+III) containing all IgG subclasses was purified by affinity chromatography on peptide-Sepharose columns. The results of ELISAs and Western blotting experiments (with the selected peptides and well-defined recombinant FVIII thrombin fragments) confirmed the reaction specificities of the affinity-purified %%%human%%% antibodies. For each IgG preparation, the isotopic subclass was also determined. In the clotting assay, several IgG preparations showed neutralising activity in a dose-dependent manner. Our observations support the recent hypothesis that FVIII inhibitors in haemophilia A and autoimmune disease may originate from the proliferation of natural FVIII-specific B-cell clones.

4/7/108
DIALOG(R)File 5:Biosis Previews(R)

0013568970 BIOSIS NO.: 200200162481
Reactivities to the Sm autoantigenic complex and the synthetic SmD1-aa83-119 peptide in systemic lupus erythematosus and other autoimmune diseases
AUTHOR: Jaekel H-P (Reprint); Klopsch T; Benkenstein B; Grobe N; Baldauf A; Schoessler W; Werle E
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JOURNAL: Journal of Autoimmunity 17 (4): p347-354 Dec., 2001 2001
MEDIUM: print
ISSN: 0896-8411
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The Sm antigenic complex is, besides dsDNA, the most important and specific autoimmune target in systemic lupus erythematosus (SLE). The population of anti-Sm Ab elicited is very heterogeneous in terms of %%%epitope%%% specificity resulting in a strong assay dependent detectability. Based on the description of a new autoantigenic target, the SmD1-aa83-119 peptide, we analysed 50 healthy persons and 205 patients with different autoimmune and other disorders with regard to their anti-Sm reactivities using different assays. The prevalence of anti-SmD1 peptide Ab and anti-Sm Ab in SLE was 36.0 (40/111) and 9.9% (11/111), respectively. The respective values obtained for non-SLE patients were 2.8 (4/144) and 5.3% (5/94). In SLE, anti-SmD1 peptide Ab are positively correlated to disease activity, nephritis and anti-dsDNA Ab. The association between reactivities of SLE samples in the traditional anti-Sm and the anti-SmD1 peptide ELISA was found to be 63.6%, contrasting markedly with the situation in non-SLE patients (no double-positive sera). SLE samples with an anti-Sm response restricted to the SmD1 peptide are completely negative in immunoblot, supporting the conformational nature of this %%%epitope%%%. Positive immunoblot reactions with the SmD1 polypeptide are not inhabitable by the synthetic SmD1-aa83-119 peptide. Comparing anti-Sm reactivities detected by ELISAs with those in immunoblot, different patterns were observed, reflecting the heterogeneous autoimmune response to this antigen. In conclusion, the anti-SmD1-aa83-119 peptide ELISA substantially completes the panel of

methods for autoantibody testing. As none of the assays presently available covers the whole spectrum of %%%epitope%%% specificities of anti-Sm Abs elicited in SLE, it does not %%%replace%%% traditional anti-Sm ELISAs.

4/7/109
DIALOG(R)File 5:Biosis Previews(R)

0013568769 BIOSIS NO.: 200200162280
%%%Epitope%%% mapping of %%%human%%% monoclonal antibodies recognizing conformational %%%epitopes%%% within HTLV type 1 gp46, employing HTLV type 1/2 envelope chimeras
AUTHOR: Hadlock Kenneth G; Yang Qing; Rowe Judy; Foung Steven K H (Reprint)
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JOURNAL: AIDS Research and Human Retroviruses 18 (1): p57-70 January 1, 2002 2002
MEDIUM: print
ISSN: 0889-2229
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LANGUAGE: English

ABSTRACT: The majority of the antibody response to HTLV-1 surface glycoprotein, gp46, is directed at conformational %%%epitopes%%%. However, the regions of HTLV-1 gp46 that contain conformational %%%epitopes%%% are poorly defined. We previously reported on %%%human%%% monoclonal antibodies (hMAbs) to conformational %%%epitopes%%% within the HTLV-1 surface glycoprotein (gp46) that inhibit HTLV-1-mediated syncytium formation (Hadlock KG, Rowe J, Perkins S, et al.: J Virol 1997; 71:5828-5840). To localize the conformational %%%epitopes%%% recognized by these antibodies, chimeric envelope proteins were constructed in which selected regions of the HTLV-1 envelope were %%%replaced%%% with the corresponding sequences from other members of the HTLV family of retroviruses. The chimeras were tested for reactivity with three hMAbs to conformational %%%epitopes%%% in HTLV-1 gp46, PRH-7A, PRH-3, and PRH-4, and one hMAb to a linear %%%epitope%%%, 0.5alpha. hMAb PRH-3 was specifically nonreactive with a chimera that %%%replaced%%% amino acids 32-36 of HTLV-1 gp46 and exhibited sharply reduced reactivity with a chimera that %%%replaced%%% amino acids 224-251 of HTLV-1 with the corresponding HTLV-2 sequence. hMAb PRH-4 was specifically nonreactive with a construct replacing amino acids 1-162 of HTLV-1 gp46 with the corresponding HTLV-2 sequence. Thus, HTLV-1 gp46 contains multiple conformational %%%epitopes%%% located in the amino-terminal portion of the protein.

4/7/110
DIALOG(R)File 5:Biosis Previews(R)

0013556970 BIOSIS NO.: 200200150481
Antagonistic effects of %%%human%%% cyclic MBP87-99 altered peptide ligands in experimental allergic encephalomyelitis and %%%human%%% T-cell proliferation
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JOURNAL: Journal of Medicinal Chemistry 45 (2): p275-283 January 17, 2002 2002
MEDIUM: print
ISSN: 0022-2623
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The immunodominant myelin basic protein (MBP) peptide comprising residues 87-99 is a self-antigen in multiple sclerosis (MS). In Lewis rats this %%%epitope%%% induces experimental allergic encephalomyelitis (EAE), a demyelinating disease of the central nervous system, and is a model of MS. Structure-activity studies have shown that Lys91 and Pro96 residues are important for encephalitogenicity. %%%Replacement%%% of Lys and/or Pro residues with Arg and/or Ala, respectively, results in suppression of EAE. A potent linear altered peptide ligand of the immunodominant sequence MBP83-99 has been selected for clinical trial (Nat. Med. 2000, 6, 1167, 1176). In the present report, two cyclic analogues, cyclo(91-99)(Ala96)MBP87-99 and cyclo(87-99)(Arg91, Ala96)MBP87-99 were designed by NMR and molecular modeling data on %%%human%%% MBP87-99 %%%epitope%%% (Val87-His-Phe-Phe-Lys-Asn-Ile-Val-Thr-Pro-Arg-Thr-Pro99) and its linear antagonist peptide analogue (Arg91, Ala96)MBP87-99. These analogues (altered peptide ligands) inhibited EAE in Lewis rats and decreased inflammation in the spinal cord. In addition, the analogue cyclo-(87-99)(Arg91, Ala96)MBP87-99 induced proliferation of %%%human%%% peripheral blood T-cells. These cyclic MBP87-99 peptide analogues may lead to the design of potent antagonist mimetics for treating MS.

4/7/111
DIALOG(R)File 5:Biosis Previews(R)

0013552996 BIOSIS NO.: 200200146507
Involvement of the C-terminal end of the prostate-specific antigen in a conformational %%%epitope%%%: Characterization by proteolytic degradation of monoclonal antibody-bound antigen and mass spectrometry
AUTHOR: Michel Sandrine; Forest Eric; Petillot Yves; Deleage Gilbert; Heuze-Vourc'h Nathalie; Courty Yves; Lascoux David; Jolivet Michel; Jolivet-Reynaud Colette (Reprint)
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JOURNAL: Journal of Molecular Recognition 14 (6): p406-413
November-December, 2001 2001
MEDIUM: print
ISSN: 0952-3499
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Prostate-specific antigen (PSA), a 237-amino acid glycoprotein, encoded by the hKLK3 gene, is widely used as a serum marker for the diagnosis and management of prostate cancer. We report here the localization of a conformational %%%epitope%%% recognized by the anti-total PSA monoclonal antibody (mAb) 11E5C6, by proteolytic degradation of mAb-bound antigen followed by mass spectrometric analyses of the peptides generated. These two technologies, combined with molecular display, allowed the identification of amino acid residues contained within three different peptides distant on the PSA sequence, but close in the PSA three-dimensional structure, that may be part of the mAb 11E5C6 %%%epitope%%%. The last four C-terminal amino acid residues are included in this %%%epitope%%%, as well as certain other C-terminal residues between Y225 and T232. The involvement of the PSA C-terminal end in the mAb 11E5C6 %%%epitope%%% was confirmed by western blotting experiments with the recombinant protein proPSA-RP1, resulting from the cloning of an alternative transcript of the hKLK3 gene, in which the PSA C-terminal end was deleted and %%%replaced%%% by another sequence. Although the anti-total PSA mAb 5D5A5 used as a control bound proPSA-RP1, mAb 11E5C6 did not. The requirement of the C-terminal end for the recognition by mAb 11E5C6 may be useful for the discrimination of PSA-related forms.

4/7/112
DIALOG(R)File 5:Biosis Previews(R)

0013547970 BIOSIS NO.: 200200141481

Hybrid papillomavirus L1 molecules assemble into virus-like particles that reconstitute conformational %%%epitopes%%% and induce neutralizing antibodies to distinct HPV types
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AUTHOR ADDRESS: Department of Microbiology and Immunology, The Milton S. Hershey Medical Center, 500 University Drive, Hershey, PA, 17033, USA** USA
JOURNAL: Virology 291 (2): p324-334 December 20, 2001 2001
MEDIUM: print
ISSN: 0042-6822
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: %%%Human%%% papillomavirus (HPV) hybrid virus-like particles (VLPs) were prepared using complementary regions of the major capsid L1 proteins of HPV-11 and -16. These hybrid L1 proteins were tested for assembly into VLPs, for presentation and mapping of conformational neutralizing %%%epitopes%%%, and as immunogens in rabbits and mice. Two small noncontiguous hypervariable regions of HPV-16 L1, when %%%replaced%%% into the HPV-11 L1 backbone, produced an assembly-positive hybrid L1 which was recognized by the type-specific, conformationally dependent HPV-16 neutralizing monoclonal antibody (N-MAb) H16.V5. Several new N-MAbs that were generated following immunization of mice with wild-type HPV-16 L1 VLPs also recognized this reconstructed VLP, demonstrating that these two hypervariable regions collectively an immunodominant %%%epitope%%%. When a set of hybrid VLPs was tested as immunogens in rabbits, antibodies to both HPV-11 and -16 wild-type L1 VLPs were obtained. One of the hybrid VLPs containing hypervariable FG and HI loops of HPV-16 L1 %%%replaced%%% into an HPV-11 L1 background provoked neutralizing activity against both HPV-11 and HPV-16. In addition, conformationally dependent and type-specific MAbs to both HPV-11 and HPV-16 L1 VLP were obtained from mice immunized with hybrid L1 VLPs. These data indicated that hybrid L1 proteins can be constructed that retain VLP-assembly properties, retain type-specific conformational neutralizing %%%epitopes%%%, can map noncontiguous regions of L1 which constitute type-specific conformational neutralizing %%%epitopes%%% recognized by N-MAbs, and trigger polyclonal antibodies which can neutralize antigenically unrelated HPV types.

4/7/113
DIALOG(R)File 5:Biosis Previews(R)

0013431486 BIOSIS NO.: 200200024997
%%%Human%%% CD4 internal antigen anti-idiotypic monoclonal antibody. Immunochemical and sequence analysis
AUTHOR: Perosa F; Luccarelli G; Prete M; Indiveri F; Dammacco F (Reprint)
AUTHOR ADDRESS: Department of Biomedical Sciences and Human Oncology (DIMO), Section of Internal Medicine, University of Bari Medical School, Piazza G. Cesare 11, I-70124, Bari, Italy**Italy
JOURNAL: Clinical and Experimental Medicine 1 (2): p81-89 June, 2001 2001
MEDIUM: print
ISSN: 1591-8890
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The mouse mAb2 16D7 recognizes the paratope of the syngeneic anti-%%%human%%% CD4 mAb HP2/6 (mAb1 of our idiotypic cascade) and mimics CD4 in xenogeneic settings in humans. Immunochemical and sequence analyses were performed to define the minimum structural requirement for this mimicry. Binding assay of mAb1 with isolated naive 16D7 H and L chains showed that only the second reacted with mAb1. Specificity was indicated by the lack of reactivity of mAb1 with the L chain of mAb2 14D6, which also recognizes mAb1-paratope. It is likely that the 16D7-L mAb1-specific %%%epitope%%% is "sequence-dependent", since fully denatured 16D7-L still reacted with mAb1. Sequence analysis of 16D7 and

mAb1 showed a high degree of homology of their VH, as both were coded by the same gene family (V/II), whereas CDR3 showed the greatest diversity. Alignment of 16D7-H CDR3 with CD4, however, produced no similarity. In contrast, analyses of the 16D7 VL sequence (XX/V) defined a CDR3 6-mer peptide with a 50% identity (83% of similarity) to the CD4 stretch 218-223. This peptide seems a suitable %%%replacement%%% for 16D7 in active immunotherapy as it did not match any protein fragment retrieved from the n-r database (NCBI) and both the peptide and the corresponding CD4 amino acid stretch are surface accessible. Based on their immunochemical profiles and similarity to CD4, four additional 16D7-derived peptides were designed for synthesis. The data indicate that CD4 mimicry by mAb2 can be obtained at the level of primary structure and provide useful information for the synthesis of peptide(s) with bioactive potential.

4/7/114
DIALOG(R)File 5:Biosis Previews(R)

0013386316 BIOSIS NO.: 200100558155
Vaccination with CTL %%%epitopes%%% that escape: An alternative approach to HIV vaccine development?
AUTHOR: O'Connor David; Allen Todd; Watkins David I (Reprint)
AUTHOR ADDRESS: Department of Pathology, Wisconsin Regional Primate Centre, University of Wisconsin, 1220 Capitol Court, Madison, WI, 53715-1299, USA **USA
JOURNAL: Immunology Letters 79 (1-2): p77-84 November 1, 2001 2001
MEDIUM: print
ISSN: 0165-2478
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: This article describes a novel approach to HIV vaccine design that is, as yet, unproven and still in preliminary development. In rhesus macaques infected with simian immunodeficiency virus (SIV), we have identified particular cellular immune responses that select for viral variants during primary infection. We speculate that the detection of viral variants with altered amino acids in CTL %%%epitopes%%% implies the successful clearance of cells harboring wild-type virus. Here, we present our rationale suggesting why such potent early CTL responses that exert an antiviral effect may be particularly attractive targets for induction by candidate vaccines. Conventional wisdom suggests that regions of the virus that are structurally and functionally important will generally be well-conserved both among clades and within an infected host. Amino acid %%%replacements%%% within these well-conserved regions should be difficult for the virus to accommodate. Therefore, these regions are traditionally considered ideal targets for vaccine induced immune responses because they are refractory to CTL escape mutations. Many examples of these regions have been identified in both HIV-1 and SIVmac (J. Immunol. 162 (1999) 3727; J. Virol. 67 (1993) 438) and have been included in candidate vaccine formulations. %%%Human%%% clinical trials testing these vaccines are currently underway. Our proposed method of vaccination with CTL %%%epitopes%%% that escape explores an alternative hypothesis. Rather than engendering responses to regions of the virus that do not escape, we reason that vaccination needs to accelerate the development of the initial immune responses that effectively select for amino acid variants during acute infection. By examining CTL escape during the acute phase, we will identify CTL responses that the virus cannot tolerate and incorporate these responses into vaccines.

4/7/115
DIALOG(R)File 5:Biosis Previews(R)

0013385926 BIOSIS NO.: 200100557765
Symmetrical homodimer of the %%%human%%% dopamine transporter revealed by cross-linking Cys306 at the extracellular end of TM6
AUTHOR: Hastrup H (Reprint); Karlin A (Reprint); Javitch J A (Reprint)

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JOURNAL: Society for Neuroscience Abstracts 27 (2): p1866 2001 2001
MEDIUM: print
CONFERENCE/MEETING: 31st Annual Meeting of the Society for Neuroscience San Diego, California, USA November 10-15, 2001; 20011110
ISSN: 0190-5295
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: There is evidence both for (Kilic and Rudnick, 2000) and against (Horiuchi et al., 2001) neurotransmitter transporters forming oligomers. We found that cross-linking the %%%human%%% dopamine transporter (DAT), heterologously expressed in EM4 cells, either with copper phenanthroline (CuP) or with the novel bifunctional reagent N,N-bis-(methanethiosulfonatoethyl)amine hydrochloride (bis-EA) increased the apparent molecular mass determined with non-reducing SDS-PAGE from apprx90 to apprx200 kDa. After cross-linking, but not before, co-expressed, differentially %%%epitope%%%-tagged DAT molecules, solubilized in Triton X-100, were co-immunoprecipitated. Thus, the 200-kDa complex was a homodimer. %%%Replacement%%% of Cys306, at the extracellular end of TM6, with Ala, prevented cross-linking. Retaining Cys306, but replacing the remaining extracellular free Cys and all Cys in the membrane domain, permitted cross-linking, indicating that the cross-link was a symmetrical disulfide between Cys306 in the two subunits. Tyramine uptake by DAT was not affected by the short S-S cross-link due to CuP or the longer crosslink due to bis-EA. The sequence GVXXGVXXA occurs in TM6 of DAT and is conserved in several types of neurotransmitter transporters. This sequence has been shown (Russ AMP; Engelman, 2000) to be a prevalent dimerization motif in membrane proteins. Mutations of the Gly in this sequence greatly diminished cell-surface expression of DAT and blocked transport completely.

0013366948 BIOSIS NO.: 200100538787
A 12-amino-acid segment, present in type s2 but not type s1 Helicobacter pylori VacA proteins, abolishes cytotoxin activity and alters membrane channel formation
AUTHOR: McClain Mark S; Cao Ping; Iwamoto Hideki; Vinion-Dubiel Arlene D; Szabo Gabor; Shao Zhifeng; Cover Timothy L (Reprint)
AUTHOR ADDRESS: Division of Infectious Diseases, Vanderbilt University School of Medicine, A3310 Medical Center North, Nashville, TN, 37232, USA **USA
JOURNAL: Journal of Bacteriology 183 (22): p6499-6508 November, 2001 2001
MEDIUM: print
ISSN: 0021-9193
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Helicobacter pylori, a gram-negative bacterium associated with gastritis, peptic ulceration, and gastric adeno-carcinoma in humans, secretes a protein toxin, VacA, that causes vacuolar degeneration of epithelial cells. Several different families of H. pylori vacA alleles can be distinguished based on sequence diversity in the "middle" region (i.e., m1 and m2) and in the 5' end of the gene (i.e., s1 and s2). Type s2 VacA toxins contain a 12-amino-acid amino-terminal hydrophilic segment, which is absent from type s1 toxins. To examine the functional properties of VacA toxins containing this 12-amino-acid segment, we analyzed a wild-type s1/m1 VacA and a chimeric s2/m1 VacA protein. Purified s1/m1 VacA from H. pylori strain 60190 induced vacuolation in HeLa and Vero cells, whereas the chimeric s2/m1 toxin (in which the s1 sequence of VacA from strain 60190 was %%%replaced%%% with the s2 sequence from strain Tx30a) lacked detectable cytotoxic activity. Type s1/m1 VacA from strain 60190 formed membrane channels in a planar lipid bilayer assay at a significantly higher rate than did s2/m1 VacA.

However, membrane channels formed by type s1 VacA and type s2 VacA proteins exhibited similar anion selectivities (permeability ratio, PCl/PNa = 5). When an equimolar mixture of the chimeric s2/m1 toxin and the wild-type s1/m1 toxin was added to HeLa cells, the chimeric toxin completely inhibited the activity of the s1/m1 toxin. Thus, the s2/m1 toxin exhibited a dominant-negative phenotype similar to that of a previously described mutant toxin, VacA-(DELTA6-27). Immunoprecipitation experiments indicated that both s2/m1 VacA and VacA-(DELTA6-27) could physically interact with a c-myc %%%epitope%%%-tagged s1/m1 VacA, which suggests that the dominant-negative phenotype results from the formation of heterooligomeric VacA complexes with defective functional activity. Despite detectable differences in the channel-forming activities and cytotoxic properties of type s1 and type s2 VacA proteins, the conservation of type s2 sequences in many H. pylori isolates suggests that type s2 VacA proteins retain an important biological activity.

4/7/117
DIALOG(R)File 5:Biosis Previews(R)

0013352238 BIOSIS NO.: 200100524077
Really old - Palaeoimmunology: Immunohistochemical analysis of extracellular matrix proteins in historic and pre-historic material
AUTHOR: Wick Georg (Reprint); Kalischnig Gerlinde; Maurer Herbert; Mayerl Christina; Mueller Pia Ulrike
AUTHOR ADDRESS: Medical School, Institute of Pathophysiology, University of Innsbruck, Fritz-Pregl-Str. 3/IV, 6020, Innsbruck, Austria**Austria
JOURNAL: Experimental Gerontology 36 (9): p1565-1579 September, 2001 2001
MEDIUM: print
ISSN: 0531-5565
DOCUMENT TYPE: Article; Literature Review
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: In this review, we summarize data concerning the respective preservation and deterioration of antigenic determinants of various collagenous and non-collagenous extracellular matrix (ECM) proteins in palaeontologic material of different ages. ECM proteins are the major quantitative constituents of mammalian organisms and were, therefore, selected as important representative proteins for these analyses. The specimens, studied by immunofluorescence and immunohistochemical techniques, included the skin of 500-1500 year-old %%%human%%% mummies from Peru, skin and striated muscle from the 5300 year-old glacier mummy ('Iceman') from Tyrol, Austria, and a 50 million year-old bat with preserved soft body parts from the fossil excavation site of Messel, Germany. In frozen sections of the former two sources, %%%epitopes%%% recognized by specific antibodies for triple-helical antigenic determinants of different types of collagen resistant against conventional proteases were preserved, while non-helical domains, as well as the non-collagenous ECM proteins, could no longer be demonstrated. The fossil bat, although showing evidence of fibrous, collagen-like structures in conventional histology, revealed no collagenous or non-collagenous ECM proteins by any technique. It later turned out that this was due to the %%%replacement%%% of the original soft parts in these fossils by lawns of bacteria. These studies introduced immunological techniques into palaeontology and opened new approaches for studying physiologically- and pathologically-altered structures in tissues of animals and humans of considerable historical age.

4/7/118
DIALOG(R)File 5:Biosis Previews(R)

0013345309 BIOSIS NO.: 200100517148
A long-term follow-up of an HIV type 1-infected patient reveals a coincidence of Nef-directed cytotoxic T lymphocyte effectors and high incidence of %%%epitope%%%-deleted variants
AUTHOR: Singh Mandaleshwar K; Janvier Genevieve; Calvez Vincent; Coulaud Pierre; Riviere Yves (Reprint)

AUTHOR ADDRESS: AIDS and Retroviruses Department, Laboratoire d'Immunopathologie Viralc, Institut Pasteur, 28 Rue du Docteur Roux, 75015, Paris, France**France
JOURNAL: AIDS Research and Human Retroviruses 17 (13): p1265-1271 September 1, 2001 2001
MEDIUM: print
ISSN: 0889-2229
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Cytotoxic T lymphocytes (CTL) play a critical role in controlling %%%human%%% immunodeficiency virus-1 (HIV-1) and simian immunodeficiency virus (SIV) infections. However, in spite of developing a strong CTL response most HIV-1-infected patients eventually progress to AIDS. Amino acid changes in CTL %%%epitope%%% have been previously described and may permit HIV to escape from CTL immune responses. The importance of CTL selection pressure in controlling the course of viral evolution in HIV-infected patient remains debatable. For over a 10-year period, we longitudinally followed a patient for bulk unstimulated effector (eCTL) and stimulated memory CTL responses (mCTL) against the viral proteins Gag, Pol, and Nef. The patient showed a strong CTL response against Nef in unstimulated peripheral blood mononuclear cells with a peak during Month 40 of the follow-up. The mCTL response was also higher against Nef than Gag and Pol. PCR amplification and nucleotide sequencing of the plasma viral variants showed a viral variant with the %%%epitope%%% deletion that was detected early during the follow-up and essentially %%%replaced%%% the wild-type virus during the peak eCTL response. These studies support the importance of Nef %%%epitope%%% deletion as a mechanism for HIV-1 escape from CTL immune pressure.

4/7/119
DIALOG(R)File 5:Biosis Previews(R)

0013328392 BIOSIS NO.: 200100500231
Determination of the binding specificity of an integral membrane protein by saturation transfer difference NMR: RGD peptide ligands binding to integrin alphaIIbbeta3
AUTHOR: Meinecke Robert; Meyer Bernd (Reprint)
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JOURNAL: Journal of Medicinal Chemistry 44 (19): p3059-3065 September 13, 2001 2001
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Saturation transfer difference (STD) NMR is a fast and versatile method to screen compound mixtures in the presence of a receptor for binding affinity and to characterize the ligand's binding %%%epitope%%%. Here we demonstrate that ligand interactions with integral membrane proteins can be investigated by STD NMR if the receptor is embedded into the lipid bilayer of a liposome. The integrin alphaIIbbeta3, also termed GPIIb-IIIa, is a platelet surface glycoprotein that plays a pivotal role in platelet aggregation and that interacts with proteins and peptides presenting the peptide recognition motif RGD. Purified %%%human%%% integrin alphaIIbbeta3 was incorporated into liposomes, and the binding of RGD peptides was analyzed by STD NMR techniques. Cyclo(RGDfV) gave STD NMR effects in the presence of liposomes containing the integrin. The magnitude of the STD effect as a function of the ligand's concentration gave a value for the dissociation constant of 30-60 muM. Adding the weakly binding RGD to the solution of cyclo(RGDfV) resulted in STD effects of the stronger ligand cyclo(RGDfV) only. This demonstrates in agreement with literature that the peptide RGD is a much weaker ligand to the integrin than the peptide cyclo(RGDfV) that largely %%%replaces%%% the RGD peptides from the binding site. The binding %%%epitope%%% of the ligand cyclo(RGDfV) was characterized by STD NMR to contain sections of

the D-Phe, the Val methyl groups, Arg alpha, beta, and gamma protons, one Hbeta of Asp, and one Halpha of Gly.

4/7/120
DIALOG(R)File 5:Biosis Previews(R)

0013321455 BIOSIS NO.: 200100493294
Gene therapy in cystic fibrosis
AUTHOR: Flotte Terence R (Reprint); Laube Beth L
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JOURNAL: Chest 120 (3 Supplement): p124S-131S September, 2001 2001
MEDIUM: print
ISSN: 0012-3692
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Theoretically, cystic fibrosis transmembrane conductance regulator (CFTR) gene %%%replacement%%% during the neonatal period can decrease morbidity and mortality from cystic fibrosis (CF). In vivo gene transfers have been accomplished in CF patients. Choice of vector, mode of delivery to airways, translocation of genetic information, and sufficient expression level of the normalized CFTR gene are issues that currently are being addressed in the field. The advantages and limitations of viral vectors are a function of the parent virus. Viral vectors used in this setting include adenovirus (Ad) and adeno-associated virus (AAV). Initial studies with Ad vectors resulted in a vector that was efficient for gene transfer with dose-limiting inflammatory effects due to the large amount of viral protein delivered. The next generation of Ad vectors, with more viral coding sequence deletions, has a longer duration of activity and elicits a lesser degree of cell-mediated immunity in mice. A more recent generation of Ad vectors has no viral genes remaining. Despite these changes, the problem of humoral immunity remains with Ad vectors. A variety of strategies such as vector systems requiring single, or widely spaced, administrations, pharmacologic immunosuppression at administration, creation of a stealth vector, modification of immunogenic %%%epitopes%%%, or tolerance induction are being considered to circumvent humoral immunity. AAV vectors have been studied in animal and %%%human%%% models. They do not appear to induce inflammatory changes over a wide range of doses. The level of CFTR messenger RNA expression is difficult to ascertain with AAV vectors since the small size of the vector relative to the CFTR gene leaves no space for vector-specific sequences on which to base assays to distinguish endogenous from vector-expressed messenger RNA. In general, AAV vectors appear to be safe and have superior duration profiles. Cationic liposomes are lipid-DNA complexes. These vectors generally have been less efficient than viral vectors but do not stimulate inflammatory and immunologic responses. Another challenge to the development of clinically feasible gene therapy is delivery mode. Early pulmonary delivery systems relied on the direct instillation of aerosolized vectors, which can result in the induction of adverse reactions because vector is delivered into the lung parenchyma. More recent studies have examined the potential for using spray technologies to target aerosolized AAV vectors to the larger central airways, thereby avoiding alveolar exposure and adverse effects. Comparisons of lung deposition with nebulized delivery of aerosol and spray delivery indicate that spraying results in a more localized deposition pattern (predominantly in the proximal airways) and significantly higher deposition fractions than nebulization. These findings could lead to more efficient and targeted lung delivery of aerosolized gene vectors in the future.

4/7/121
DIALOG(R)File 5:Biosis Previews(R)

0013310432 BIOSIS NO.: 200100482271
Agonist monoclonal antibodies to %%%human%%% trkC receptor map to %%%epitopes%%% overlapping with NT-3 binding site

AUTHOR: Hongo J (Reprint); Bald L (Reprint); Intintoli A; Poulsen K (Reprint); O'Connell L (Reprint); Lowman H; Nishimura M; Fendly B (Reprint); Presta L (Reprint); Sadick M; Devaux B (Reprint); Shelton D L (Reprint)
AUTHOR ADDRESS: Immunology, Genentech, Inc, South San Francisco, CA, USA** USA
JOURNAL: Society for Neuroscience Abstracts 27 (1): p356 2001 2001
MEDIUM: print
CONFERENCE/MEETING: 31st Annual Meeting of the Society for Neuroscience San Diego, California, USA November 10-15, 2001; 20011110
ISSN: 0190-5295
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Large diameter, myelinated sensory neurons express trkC, the high affinity receptor for neurotrophin-3 (NT-3), and mediate proprioception and vibration sense. We have generated a panel of agonist monoclonal antibodies (MAbs) to %%%human%%% trkC, capable of inducing tyrosine phosphorylation of trkC and promoting neurite outgrowth of PC12 cells expressing trkC. These MAbs, unlike the NT-3 ligand, do not cross react with any of the other %%%human%%% trk receptors. Only two of them show cross reactivity with the rat trkC homolog. Using domain %%%replacement%%% trkC mutants, we demonstrated that most agonist antibodies mapped to trkC domain 5. Moreover, fine %%%epitope%%% mapping of these MAbs identified 5 contact residues located in loops AB and EF of trkC domain 5, residues previously shown to be involved in binding of the ligand NT-3. Our results demonstrate that some of these agonist MAbs are capable of triggering activation of trkC almost as efficiently as the NT-3 ligand. Moreover, an extensive screen identified MAbs binding %%%epitopes%%% converging to domain 5. This suggests that binding to this specific domain strongly favors agonist activity.

4/7/122
DIALOG(R)File 5:Biosis Previews(R)

0013295513 BIOSIS NO.: 200100467352
A first-tier rapid assay for the serodiagnosis of Borrelia burgdorferi infection
AUTHOR: Gomes-Solecki Maria J C; Wormser Gary P; Persing David H; Berger Bernard W; Glass John D; Yang Xiaohua; Dattwyler Raymond J (Reprint)
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JOURNAL: Archives of Internal Medicine 161 (16): p2015-2020 September 10, 2001 2001
MEDIUM: print
ISSN: 0003-9926
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Background: The present recommendation for the serologic diagnosis of Lyme disease is a 2-tier process in which a serum sample with a positive or equivocal result by an enzyme-linked immunosorbent assay (ELISA) or immunofluorescent assay is then followed by supplemental testing by Western blot. Our laboratory has developed recombinant chimeric proteins composed of key Borrelia %%%epitopes%%%. These novel antigens are consistent and are easily standardized. Methods: We adapted these recombinant proteins into a new immunochromatographic format that can be used as a highly sensitive and specific first-tier assay that can be used to %%%replace%%% the ELISA or immunofluorescent assay. Results: This rapid test was equally sensitive (P>.05) and more specific (P<.05) than a frequently used commercial whole cell ELISA. The overall clinical accuracy achieved on agreement studies among 3 Lyme research laboratories on clinically defined serum panels was shown to be statistically equivalent to the commercial ELISA. The assay can detect anti-Borrelia burgdorferi antibodies in either serum or whole blood. Conclusion: This sensitive and specific rapid assay, which is suited for the physician's office, streamlines the 2-tier system by allowing the physician to

determine if a Western blot is necessary at the time of the initial office visit.

4/7/123
DIALOG(R)File 5:Biosis Previews(R)

0013293753 BIOSIS NO.: 200100465592
Effect of preoperative oral immune-enhancing nutritional supplement on patients at high risk of infection after cardiac surgery: A randomised placebo-controlled trial
AUTHOR: Tepaske Robert (Reprint); te Velthuis Henk; Oudemans-van Straaten Heleen M; Heisterkamp Siem H; van Deventer Sander J H; Ince Can; Eysman Leon; Kesecioglu Jozef
AUTHOR ADDRESS: Department of Intensive Care, Academic Medical Centre, C3-324, 1100DD, Amsterdam ZO, Netherlands**Netherlands
JOURNAL: Lancet (North American Edition) 358 (9283): p696-701 1 September, 2001 2001
MEDIUM: print
ISSN: 0099-5355
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Background: Elderly patients and those with poor ventricular function have increased morbidity and mortality rates when undergoing surgery. We aimed to ascertain whether an oral immune-enhancing nutritional supplement could improve preoperative host defence, and subsequently lower postoperative infections and organ dysfunction in patients undergoing elective cardiac surgery who are at high risk of infection. Methods: In this prospective, randomised, double-blind, placebo-controlled study, we randomly assigned 50 patients who were scheduled to undergo coronary artery bypass to receive either an oral immune-enhancing nutritional supplement containing L-arginine, omega3 polyunsaturated fatty acids, and yeast RNA (n=25), or a control (n=25) for a minimum of 5 days. Patients were included if they were aged 70 years or older, or had an ejection fraction of less than 0.4, or were scheduled to undergo mitral valve %%%replacement%%%. The main outcome was preoperative host defence (delayed-type hypersensitivity response to recall antigens, expression of HLA-DR %%%epitopes%%% on monocytes, and concentration of interleukin 6 in plasma). Analysis was per protocol. Findings: Five patients (two in the treatment group) were excluded because they did not take the minimum dose. Preoperative expression of HLA-DR %%%epitopes%%% on monocytes was significantly higher in patients given the study treatment (109% (95% CI 92-128)) than those given the control (69% (58-82)) compared with baseline (100%) (p=0.02, repeated measures ANOVA). However, concentration of interleukin 6 was significantly lower in the treatment group (0.90 pg/L (0.69-1.18)) than in the control group (1.94 pg/L (1.45-2.59)) (p=0.032, repeated measures ANOVA). Additionally, delayed-type hypersensitivity response to recall antigens improved preoperatively and remained better until hospital discharge. Interpretation: Intake of an oral immune-enhancing nutritional supplement for a minimum of 5 days before surgery can improve outlook in high-risk patients who are undergoing elective cardiac surgery.

4/7/124
DIALOG(R)File 5:Biosis Previews(R)

0013291507 BIOSIS NO.: 200100463346
Symmetrical dimer of the %%%human%%% dopamine transporter revealed by cross-linking Cys-306 at the extracellular end of the sixth transmembrane segment
AUTHOR: Hastrup Hanne; Karlin Arthur; Javitch Jonathan A (Reprint)
AUTHOR ADDRESS: Center for Molecular Recognition, College of Physicians and Surgeons, Columbia University, 630 West 168th Street, New York, NY, 10032, USA**USA
JOURNAL: Proceedings of the National Academy of Sciences of the United States of America 98 (18): p10055-10060 August 28, 2001 2001

MEDIUM: print
ISSN: 0027-8424
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: There is evidence both for and against Na+- and Cl--dependent neurotransmitter transporters forming oligomers. We found that cross-linking the %%%human%%% dopamine transporter (DAT), which is heterologously expressed in %%%human%%% embryonic kidney 293 cells, either with copper phenanthroline (CuP) or the bifunctional reagent bis-(2-methanethiosulfonatoethyl)amine hydrochloride (bis-EA) increased the apparent molecular mass determined with non-reducing SDS/PAGE from apprxeq85 to apprxeq195 kDa. After cross-linking, but not before, coexpressed, differentially %%%epitope%%%-tagged DAT molecules, solubilized in Triton X-100, were coimmunoprecipitated. Thus, the 195-kDa complex was a homodimer. Cross-linking of DAT did not affect tyramine uptake. %%%Replacement%%% of Cys-306 with Ala prevented cross-linking. %%%Replacement%%% of all of the non-disulfide-bonded cysteines in the extracellular and membrane domains, except for Cys-306, did not prevent cross-linking. We conclude that the cross-link is between Cys-306 at the extracellular end of TM6 in each of the two DATs. The motif GVXXGVXXA occurs at the intracellular end of TM6 in DAT and is found in a number of other neurotransmitter transporters. This sequence was originally found at the dimerization interface in glycophorin A, and it promotes dimerization in model systems. Mutation of either glycine disrupted DAT expression and function. The intracellular end of TM6, like the extracellular end, is likely to be part of the dimerization interface.

4/7/125
DIALOG(R)File 5:Biosis Previews(R)

0013270102 BIOSIS NO.: 200100441941
The GOR gene product cannot cross-react with hepatitis C virus in humans
AUTHOR: Koike R (Reprint); Iizuka T; Watanabe T; Miyasaka N
AUTHOR ADDRESS: First Department of Internal Medicine, Tokyo Medical and Dental University, 1-5-45 Yushima, Bunkyo-ku, Tokyo, 113, Japan**Japan
JOURNAL: Clinical and Experimental Immunology 124 (3): p429-434 June, 2001
2001
MEDIUM: print
ISSN: 0009-9104
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: GOR (GOR47-1) is an %%%epitope%%% thought to be a host-derived antigen cross-reactive with hepatitis C virus (HCV) since it was isolated from a cDNA library of host animals reactive with sera of HCV-positive patients. An enzyme immunosorbent assay (ELISA) using this %%%epitope%%% as antigen is of sufficient sensitivity and specificity for screening patients with HCV. However, the relationship between GOR47-1 %%%epitope%%% and autoimmune phenomena associated with HCV infection or autoimmune hepatitis is controversial. Here we isolated the %%%human%%% GOR gene and found that the GOR47-1 %%%epitope%%% was not translated in humans due to a single base %%%replacement%%% from chimpanzee. Furthermore, we found some patients who had antibodies against another %%%epitope%%%, which is translated (GOR1-125) in humans, although there was no correlation between the existence of anti-GOR47-1 or anti-GOR1-125 Ab and autoimmune phenomena. Serum IgG levels did not influence the titres of these antibodies. Taken together with the results of several other studies, our finding that the GOR47-1 %%%epitope%%% cannot be translated into a protein suggests that there is little relationship between autoimmunity and the GOR gene product in %%%human%%% beings. We also discuss here the possible mechanism of cross-reactivity between HCV and the GOR gene product.
? ds

Set	Items	Description
S1	2	HUMANIZED(5W)TOXIN?

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S2          4   HUMANIZED(5W)ENZYME?
S3        957   EPITOP? AND REPLACE?
S4        496   S3 AND HUMAN?
S5          0   SSSSSS
? s epitope swap
      S6       0  EPITOPE SWAP
? s enzyme and epitope and replace?
          648759  ENZYME
           34238  EPITOPE
          155670  REPLACE?
      S7      91  ENZYME AND EPITOPE AND REPLACE?
? t s7/7/50-60

 7/7/50
DIALOG(R)File   5:Biosis Previews(R)
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0010909175   BIOSIS NO.: 199799543235
Cystic fibrosis transmembrane conductance regulator: The first nucleotide
  binding fold targets the membrane with retention of its ATP binding
  function
AUTHOR: Ko Young Hee; Delannoy Michael; Pedersen Peter L (Reprint)
AUTHOR ADDRESS: Dep. Biol. Cell Biol. Chem., Johns Hopkins Univ. Sch. Med.,
  725 N. Wolfe St., Baltimore, MD 21205-2185, USA**USA
JOURNAL: Biochemistry  36 (16): p5053-5064 1997 1997
ISSN: 0006-2960
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Most cases of cystic fibrosis are caused by a single deletion
  mutation (DELTA-F508) within the first nucleotide binding fold (NBF1) of
  the CFTR protein (cystic fibrosis transmembrane conductance regulator).
  NBF1 is classically defined as amino acid residues phenylalanine 433
  through serine 589, encoded by exons 10-12, and only part of exon 9, of
  the CFTR gene. This assignment is based on sequence homology of this
  region of the CFTR protein with that of other nucleotide binding
  proteins. Here, we report that when the complete modular unit encoded
  precisely by exons 9-12 is expressed in Escherichia coli as glycine 404
  through serine 589, i.e., as (G404-N432)NBF1 or as
  DELTA-F508(G404-N432)NBF1, the resultant proteins target the cytoplasmic
  membrane. Significantly, (G404-N432)NBF1 is readily labeled from the
  outside of intact E. coli spheroplasts with the water soluble, membrane
  impermeable probe Biotin-X-NHS,
  sulfosuccinimidyl-6-(biotinamido)-hexanoate. Similar findings were
  observed with the disease causing mutant DELTA-F508(G404-N432)NBF1. Three
  different control experiments which involved (1) assays for known
  cytosolic E. coli enzymes, (2) immuno-gold electron microscopy with
  antibody having an %%%epitope%%% for the biotin moiety, and (3) tests for
  biotinylation of the cytosolic component, %%%Enzyme%%% 1 of the glucose
  phosphotransferase system, demonstrated that the spheroplasts used in
  this study are neither leakly nor permeable to Biotin-X-NHS. In addition,
  membrane-associated (G404-N432)NBF1, upon solubilization with Triton
  X-100, was found to bind to an ATP-agarose column and be released
  therefrom by elution with ATP, emphasizing retention of a native-like
  structure. In sharp contrast, NBF1 localizes to the cytosol when the
  (G404-N432)-N-terminal region is %%%replaced%%% with the maltose binding
  protein. The novel findings reported here implicate a role of the
  N-terminal region of NBF1 in its subcellular localization and are
  directly relevant to our understanding of the membrane structure,
  function, and trafficking of CFTR.


 7/7/51
DIALOG(R)File   5:Biosis Previews(R)
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0010843447   BIOSIS NO.: 199799477507
The HSD-hCG vaccine prevents pregnancy in women: Feasibility study of a
  reversible safe contraceptive vaccine
AUTHOR: Talwar G P (Reprint); Singh Om; Gupta S K; Hasnain S E; Pal R;
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Majumdar S S; Vrati S; Mukhopadhay A; Srinivasan J; Deshmukh U; Ganga S; Mandokhot A; Gupta A
AUTHOR ADDRESS: International Centre for Genetic Eng. and Biotechnology, New Delhi 110067, India**India
JOURNAL: American Journal of Reproductive Immunology 37 (2): p153-160 1997 1997
ISSN: 1046-7408
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: PROBLEM: To develop a vaccine for reversible control of fertility in women. MATERIALS AND PROTOCOLS: Purified beta subunit of hCG annealed to purified alpha subunit of ovine LH linked chemically to tetanus toxoid (TT) and diphtheria (DT); vaccine employed at 300 mu-g gonadotropin equivalent per injection adsorbed on alhydrogel with 1 mg SPLPS added in the first injection; Phase I safety trials in 47 women with elective tubal ligation; Phase II efficacy studies in 148 proven fertile women (2 children), sexually active, desirous of family planning using IUD; IUD removed when anti-hCG titres exceed 50 ng/ml hCG bioneutralization capacity; boosters given to maintain above threshold antibody levels; post coital tests conducted in 8 volunteers; sera of protected women analysed for immuno-determinants recognized by competitive %%%enzyme%%% immunoassays employing a panel of monoclonal antibodies and by direct binding to synthetic peptides; recombinant vaccines expressing beta-hCG as a secreted product or as a fused protein anchored on membrane. RESULTS: Immunization was well tolerated with no significant changes in endocrine, metabolic and hematological indices. Normal ovulatory cycles were maintained as indicated by menstrual regulation. The vaccine was highly effective in preventing pregnancy (1 pregnancy in 1224 cycles) at and above antibody titres of 50 ng/ml. Antibodies declined in course of time in absence of boosters, with conceptions occurring below 35 ng/ml titres indicating regain of fertility. Ability of antibodies to prevent pregnancy was confirmed by post coital tests. High avidity (10-10 M-1) and other characteristics of antibodies generated by the vaccine are described and compared with those induced by two other hCG vaccines having undergone Phase I trials. The antibody response of the HSD vaccine in humans is characterized predominantly to an %%%epitope%%% recognized by the monoclonals 206 and P-3W-80. The antibodies had low or no reactivity with the carboxy terminal peptide and 38-57 region peptide. Live recombinant vaccines expressing beta-hCG as a membrane anchored peptide generated antibody response to hCG in all animals following a single injection. CONCLUSIONS: Reversible fertility control is feasible with the HSD-hCG vaccine without impairment of ovulation or disturbance of menstrual regularity. Suggestions have been made for further optimization of the vaccine, which include %%%replacement%%% of TT and DT by a panel of T non B determinants communicating with the entire MHC spectrum and development of recombinant vaccine expressing beta-hCG along with membrane anchored carrier.

7/7/52
DIALOG(R)File 5:Biosis Previews(R)

0010836572 BIOSIS NO.: 199799470632
Alteration in apolipoprotein A-I 22-Mer repeat order results in a decrease in lecithin:cholesterol acyltransferase reactivity
AUTHOR: Sorci-Thomas Mary G (Reprint); Curtiss Linda; Parks John S; Thomas Michael J; Kearns Mary W
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JOURNAL: Journal of Biological Chemistry 272 (11): p7278-7284 1997 1997
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Apolipoprotein A-I contains eight 22-amino acid and two 11-amino acid tandem repeats that comprise 80% of the mature protein. These repeating units are believed to be the basic motif responsible for lipid

binding and lecithin:cholesterol acyltransferase (LCAT) activation. Computer analysis indicates that despite a fairly high degree of compositional similarity among the tandem repeats, significant differences in hydrophobic and amphipathic character exist. Our previous studies demonstrated that deletion of repeat 6 (143-164) or repeat 7 (165-186) resulted in a 98-99% reduction of LCAT activation as compared with wild-type apoA-I. To determine the effects of substituting one of these repeats with a more hydrophobic repeat we constructed a mutant apoA-I protein in which residues 143-164 (repeat 6) were %%%replaced%%% with repeat 10 (residues 220-241). The cloned mutant protein, 10F6 apoA-I, was expressed and purified from an Sf-9 cell baculoviral system and then analyzed using a number of biophysical and biochemical techniques. Recombinant complexes prepared at a 100:5:1 molar ratio of L-alpha-dimyristoylphosphatidylcholine: cholesterol:wild-type or 10F6 apoA-I showed a doublet corresponding to Stokes diameters of 114 and 108 ANG on nondenaturing 4-30% polyacrylamide gel electrophoresis. L-alpha-Dimyristoylphosphatidylcholine 10F6 apoA-I complexes had a 5-6-fold lower apparent V-max/apparent K-m as compared with wild-type apoA-I containing particles. As expected, monoclonal antibody %%%epitope%%% mapping of the lipid-free and lipid-bound 10F6 apoA-I confirmed that a domain expressed between residues 143 and 165 normally found in wild-type apoA-I was absent. The region between residues 119 and 144 in 10F6 apoA-I showed a marked reduction in monoclonal antibody binding capacity. Therefore, we speculate that the 5-6-fold lower LCAT reactivity in 10F6 compared with wild-type apoA-I recombinant particles results from increased stabilization within the 121-165 amino acid domain due to more stable apoprotein helix phospholipid interactions as well as from conformational alterations among adjacent amphipathic helix repeats.

7/7/53
DIALOG(R)File 5:Biosis Previews(R)

0010774057 BIOSIS NO.: 199799408117
Escherichia coli dimethylallyl diphosphate:tRNA dimethylallyltransferase: A binding mechanism for recombinant %%%enzyme%%%
AUTHOR: Moore Jeffrey A; Poulter C Dale (Reprint)
AUTHOR ADDRESS: Dep. Chem., Univ. Utah, Salt Lake City, UT 84112, USA**USA
JOURNAL: Biochemistry 36 (3): p604-614 1997 1997
ISSN: 0006-2960
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Escherichia coli dimethylallyl diphosphate:tRNA dimethylallyltransferase (DMAPP-tRNA transferase) catalyzes the first step in the biosynthesis of the hypermodified A37 residue in tRNAs that read codons beginning with uridine. The %%%enzyme%%%, encoded by the miaA gene, was overproduced and purified to apparent homogeneity in three steps by ion-exchange (DE52 and Mono-Q) and size exclusion chromatography. Affinity-tagged DMAPP-tRNA transferase containing a C-terminal tripeptide alpha-tubulin %%%epitope%%% also was overproduced and purified to apparent homogeneity in two steps by ion-exchange and immunoaffinity chromatography. Addition of the C-terminal tripeptide alpha-tubulin %%%epitope%%% to DMAPP-tRNA transferase did not affect the activity of the %%%enzyme%%%. Undermodified tRNA-Phe used as substrate in the DMAPP-tRNA transferase-catalyzed reaction was isolated and purified from an overexpressing clone in a miaA deficient strain of E. coli. Active recombinant E. coli DMAPP-tRNA transferase is monomeric. The %%%enzyme%%% transferred the dimethylallyl moiety of DMAPP to A37, located adjacent to the anticodon in undermodified tRNA-Phe. The %%%enzyme%%% required Mg-2+ for activity and exhibited a broad pH optimum. Michaelis constants for tRNA-Phe and DMAPP are 96 +- 11 nM and 3.2 +- 0.5 mu-M, respectively, and V-max = 0.83 +- 0.02 mu-mol min-1 mg-1. DMAPP-tRNA transferase bound tRNA-Phe with a dissociation constant of 5.2 +- 1.2 nM. In contrast, DMAPP did not bind to the %%%enzyme%%% in the absence of tRNA. However, DMAPP was bound with a dissociation constant of 3.4 +- 0.6 mu-M in the presence of a minihelix analogue of the anticodon stem-loop of tRNA-Phe where the base corresponding to A37 was %%%replaced%%% by inosine. These results suggest an ordered

sequential mechanism for substrate binding.

7/7/54
DIALOG(R)File 5:Biosis Previews(R)

0010597475 BIOSIS NO.: 199699231535
Proline-rich tandem repeats of antibody complementarity-determining regions bind and neutralized human immunodeficiency virus type 1 particles
AUTHOR: Fontenot J Darrell; Zacharopoulos Vanaja R; Phillips David M (Reprint)
AUTHOR ADDRESS: Population Council, 1230 York Ave., New York, NY 10021, USA **USA
JOURNAL: Journal of Virology 70 (10): p6557-6562 1996 1996
ISSN: 0022-538X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The proline-rich tandem repeat domain of human mucin MUC1 forms an extended structure containing large repeating loops that are crested by a turn. We show that the repeating-loop structure of MUC1 can be %%%replaced%%% by an antibody complementarity-determining region loop of a human immunodeficiency virus type 1 (HIV-1)-specific neutralizing antibody to create a chimeric, multivalent, mucin-like, anti-HIV-1 compound. We used 8 residues of an antibody molecule to %%%replace%%% 8 of 20 residues of the MUC1 tandem-repeat sequence. The antiviral peptide discussed here contains three copies of a 20-residue tandem repeat, (IYYDYEEDPAPGSTAPPAHG)-3, for a total of 60 residues. We demonstrate that the mucin-antibody chimera retains the binding specificity of the parent antibody (monoclonal antibody F58), GPGR of the HIV-1 gp120 V3 neutralizing %%%epitope%%%, and the ability to neutralize virus particles. In inhibition %%%enzyme%%%-linked immunosorbent assay, the mucin-antibody chimeric peptide could inhibit 71 to 84% of binding to a V3 loop peptide by monoclonal antibodies known to be specific for GPGR in the V3 loop. The mucin-antibody chimeric peptide could also inhibit monoclonal antibody binding to native gp120 captured from virus particles. In addition, the chimeric peptide neutralized the homologous HIV-IIIB virus in a standard neutralization assay. The methods of antiviral peptide design and construction presented here are general and theoretically limited only by the size of the antibody repertoire. This approach could be used to synthesize peptides for a variety of therapeutic applications.

7/7/55
DIALOG(R)File 5:Biosis Previews(R)

0010447891 BIOSIS NO.: 199699081951
Crystal structure of human trypsin 1: Unexpected phosphorylation of Tyr151
AUTHOR: Gaboriaud Christine (Reprint); Serre Laurence; Guy-Crotte Odette; Forest Eric; Fontecilla-Camps Juan-Carlos
AUTHOR ADDRESS: Lab. de Cristallographie et Cristallogenese des Proteines, Inst. de Biologie Structurale Jean-Pierre Ebel, 41 Avenue des Martyrs, 30827 Grenoble Cedex 1, France**France
JOURNAL: Journal of Molecular Biology 259 (5): p995-1010 1996 1996
ISSN: 0022-2836
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The X-ray structure of human trypsin 1 has been determined in the presence of diisopropyl-phosphofluoridate by the molecular %%%replacement%%% method and refined at a resolution of 2.2 ANG to an R-factor of 18%. Crystals belong to the space group P4, with two independent molecules in the asymmetric unit packing as crystallographic tetramers. This study was performed in order to seek possible structural peculiarities of human trypsin 1, suggested by some striking differences in its biochemical behavior as compared to other trypsins of mammalian

species. Its fold is, in fact, very similar to those of the bovine, rat and porcine trypsins, with root-mean-square differences in the 0.4 to 0.6 ANG range for all 223 C-alpha positions. The most unexpected feature of the human trypsin 1 structure is in the phosphorylated state of tyrosine residue 151 in the present X-ray study. This feature was confirmed by mass spectrometry on the same inhibited sample and also on the native %%%enzyme%%%. This phosphorylation strengthens the outstanding clustering of highly negative or highly positive electrostatic surface potentials. The peculiar inhibitory behaviour of pancreatic secretory trypsin inhibitors of the Kazal type on this %%%enzyme%%% is discussed as a possible consequence of these properties. A charged surface loop has also been interpreted as an %%%epitope%%% site recognised by a monoclonal antibody specific to human trypsin 1.

7/7/56
DIALOG(R)File 5:Biosis Previews(R)

0010031378 BIOSIS NO.: 199598499211
Presentation of HIV V3 loop epitopes for enhanced antigenicity, immunogenicity and diagnostic potential
AUTHOR: Fontenot J Darrell (Reprint); Vancott Tom C; Parekh Bharat S; Pau Chou-Pong; George J Richard; Birx Debra L; Zolla-Pazner Susan; Gorny Miroslaw K; Gatewood Jose M
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JOURNAL: AIDS (Philadelphia) 9 (10): p1121-1129 1995 1995
ISSN: 0269-9370
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Objective: To evaluate the immunological properties of a panel of human mucin MUC1/HIV V3 loop chimeras. Design: The immunodominant %%%epitope%%% of MUC1 (APDTR) was found to be structurally isomorphous with the tip of the principle neutralizing determinant (PND) of HIV-1 (MN) (GPGRA). A panel of 120 residue, six tandem repeat (TR) and 60 residue, three TR chimeric antigens were constructed in which the repeating MUC1 %%%epitope%%% is %%%replaced%%% by HIV-1 PND. Each 20 residue TR contains one PND %%%epitope%%%. The PND of HIV-1 is presented in the native beta-turn conformation at the crest of each repeating knob structure of the mucin-like protein. Methods: The antigenicity of the chimeric antigens were compared using %%%enzyme%%%-linked immunosorbent assay (ELISA) and HIV-infected patient sera. Structural effects of antibody-antigen interactions were determined using surface plasmon resonance, with human monoclonal antibodies, chimeric antigens and the cyclic and linear V3 loops. Immunogenicity of three versus six TR was measured in mice. Results: Nine residues of the HIV PND substituted into the mucin backbone were equivalent to the 36 residue cyclic V3 loop in ELISA. The 120 residue antigens induced high titer, immunoglobulin (Ig) M and IgG, and HIV-specific antibodies in mice. Conclusions: MUC1/V3 chimeras efficiently detect HIV-specific antibodies in patient sera. Multivalent presentation of the PND is advantageous for higher affinity antibody-antigen interactions and for inducing HIV-specific IgM and IgG antibodies.

7/7/57
DIALOG(R)File 5:Biosis Previews(R)

0009892232 BIOSIS NO.: 199598360065
An approach for an immunoaffinity AIDS sensor using the conservative region of the HIV envelope protein (gp41) and its monoclonal antibody
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JOURNAL: Biosensors and Bioelectronics 10 (5): p477-483 1995 1995
ISSN: 0956-5663

DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A monoclonal antibody for the conservative region of gp41, which is one of the HIV envelope proteins, was produced. The antigen determining site of gp41 was examined using the %%%epitope%%% mapping technique, followed by an %%%enzyme%%% linked immunosorbent assay. Some peptides had comparable affinities for the monoclonal antibody, but the peptide EGIEE, having a slightly weaker immunoaffinity than gp41, was the most preferable for the construction of an immunoaffinity AIDS sensor. For the detection of gp41, EGIEE was labelled with catalase and used as a mimic antigen; it was bound to the antibody present on an immuno-membrane and, due to the %%%replacement%%% reaction of the mimic antigen by gp41, indirect quantitative measurement of gp41 was possible using an oxygen electrode. Anti-gp41 antibody was also detected using a mimic antibody, which was chemically modified with polyethylene glycol. An immunoaffinity AIDS sensor was constructed using the mimic molecules which were tailored to have a suitable immunoaffinity for HIV antigen and/or antibody.

7/7/58
DIALOG(R)File 5:Biosis Previews(R)

0009765830 BIOSIS NO.: 199598233663
Acceptor specificity of different length constructs of human recombinant alpha-1,3/4-fucosyltransferase: %%%Replacement%%% of the stem region and the transmembrane domain of fucosyltransferase V by protein A results in an %%%enzyme%%% with GDP-fucose hydrolyzing activity
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JOURNAL: Journal of Biological Chemistry 270 (15): p8712-8722 1995 1995
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The acceptor specificity of recombinant full-length, membrane-bound fucosyltransferases, expressed in COS-7 cells, and soluble, protein-A chimeric forms of alpha-1,3-fucosyltransferase (Fuc-T) III, Fuc-TIV, and Fuc-TV was analyzed toward a broad panel of oligosaccharide, glycolipid, and glycoprotein substrates. Our results on the full-length enzymes confirm and extend previous studies. However, chimeric Fuc-Ts showed increased activity toward glycoproteins, whereas chimeric Fuc-TIII and Fuc-TV had a decreased activity with glycosphingolipids, compared to the full-length enzymes. Unexpectedly, chimeric Fuc-TV exhibited a GDP-fucose hydrolyzing activity. In substrates with multiple acceptor sites, the preferred site of fucosylation was identified. Fuc-TIII and Fuc-TV catalyzed fucose transfer exclusively to OH-3 of glucose in lacto-N-neotetraose and lacto-N-tetraose, respectively, as was demonstrated by 1H NMR spectroscopy. Thin layer chromatography immunostaining revealed that FucT-IV preferred the distal GlcNAc residue in nLc-6Cer, whereas Fuc-TV preferred the proximal GlcNAc residue. Incubation of Fuc-TIV or Fuc-TV with VI-3NeuAcnLc-6Cer resulted in products with the sialyl-Lewis-x %%%epitope%%% as well as the VIM-2 structure. To identify polar groups on acceptors that function in %%%enzyme%%% binding, deoxygenated substrate analogs were tested as acceptors. All three Fuc-Ts had an absolute requirement for a hydroxyl at C-6 of galactose in addition to the accepting hydroxyl at C-3 or C-4 of GlcNAc.

7/7/59
DIALOG(R)File 5:Biosis Previews(R)

0009610570 BIOSIS NO.: 199598078403
Analogues of peptide 9-21 of glycoprotein D of herpes simplex virus and

their binding to group VII monoclonal antibodies
AUTHOR: Welling-Wester S (Reprint); Feijlbrief M; Koedijk D G A M; Drijfhout J W; Weijer W J; Scheffer A J; Welling G W
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JOURNAL: Archives of Virology 138 (3-4): p331-340 1994 1994
ISSN: 0304-8608
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Several analogues of the amino acid sequence of peptide 9-21 of glycoprotein D of herpes simplex virus type 1 (HSV-1) were synthesized and investigated for reactivity with different group VII monoclonal antibodies. Mabs LP14, ID3, 170, HD4, A16, E-II-24 and E-V-10, in a competition %%%enzyme%%%-linked immunosorbent assay (ELISA). %%%Replacement%%% of Arg at position 16 by His resulted in a loss of binding with the group VII Mabs. Substitution of Pro at residue 14 by Leu gave a reduced binding for a number of Mabs and loss of binding for Mab A16. Substitution of Lys at position 10 by Glu gave reduced binding for three out of the seven Mabs. In addition substitutions of Met at position 11 by norleucine and oxidized Met were studied. The boundaries of the %%%epitope%%% cluster were mapped by studying synthetic variants of peptide 9-21 which were shorter either at the C-terminus or at the N-terminus, or both. Peptides 10-18 and peptide 9-17 were the shortest peptides, which were still reactive with the group VII Mabs. Mab HD4 requires the N-terminus of peptide 9-21 for effective binding, while for finding of other Mabs contribution of the residues in the C-terminal part of this peptide is more important.

7/7/60
DIALOG(R)File 5:Biosis Previews(R)

0009496609 BIOSIS NO.: 199497517894
Erwinia chrysanthemi L-asparginase: %%%Epitope%%% mapping and production of antigenically modified enzymes
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JOURNAL: Biochemical Journal 302 (3): p921-927 1994 1994
ISSN: 0264-6021
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: This study shows that the antigenicity of Erwinia chrysanthemi Lasparaginase can be reduced by site-directed mutagenesis. Ten B-cell epitopes of the %%%enzyme%%% were identified using synthetic hexapeptides and polyclonal antisera from rabbits and mice. The region 282GIVPPDEELPG-292 near the C-terminus was an immunodominant %%%epitope%%%. Binding of two hexapeptides (283IVPPDE-288 and 287DEELPG-291) to the antibodies was dependent on Pro-285, and Pro-286, since their %%%replacement%%% by almost any other amino acid resulted in reduced binding. The other residues were less important for binding the antibodies, as binding was relatively unaffected by amino acid substitutions. Three site-directed mutant enzymes, P285T (proline-285 fwdarw threonine etc.), P286Q and E288A, were expressed in Escherichia coli. The purified enzymes had subunit M, values of 35000. The pI values of P285T, P286Q and the wild-type enzymes were 8.6, and that for the mutant E288A was 9.2. The k-cat and K-m values for the mutants P286Q and E288A with L-asparagine and L-glutamine were comparable with those of the wild-type %%%enzyme%%%. The K-m values for the mutant P285T with both substrates was similar to that of the wild-type %%%enzyme%%%, whereas the k,al. was reduced by 2-fold with L-asparagine and by 4-fold with L-glutamine. The change proline fwdarw threonine reduced the antigenicity of the %%%enzyme%%% by 8-fold, as shown in sandwich e.l.i.s.a.s. using monoclonal antibodies raised against the wild-type %%%enzyme%%%.

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8/7/1
DIALOG(R)File 5:Biosis Previews(R)

0015334662 BIOSIS NO.: 200510029162
Epitopes recognized by a nonautoreactive murine anti-N-propionyl meningococcal group B polysaccharide monoclonal antibody
AUTHOR: Moe Gregory R (Reprint); Dave Apurva; Granoff Dan M
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JOURNAL: Infection and Immunity 73 (4): p2123-2128 APR 05 2005
ISSN: 0019-9567
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The capsular polysaccharide of Neisseria meningitidis group B (MBPS) is a polymer of alpha (2 -> 8) N-acetyl neuraminic acid. The polysaccharide is chemically identical to an autoantigen, polysialic acid (PSA), and is a poor immunogen, even when conjugated to protein carriers. Immunization of mice with MBPS-protein conjugate vaccines, in which N-acetyl groups have been %%%replaced%%% by propionyl groups (N-Pr MBPS), elicits serum bactericidal antibodies. A subpopulation of these antibodies do not cross-react with %%%human%%% PSA. The reasons for the increased immunogenicity of N-Pr MBPS and the antigenic targets of the bactericidal nonautoreactive antibodies are unknown. In this study, we investigated the antigenic targets of a protective murine monoclonal antibody (MAb) prepared against a N-Pr MBPS-tetanus toxoid conjugate vaccine. Binding of the MAb to N-Pr MBPS (as demonstrated by an %%%enzyme%%%-linked immunosorbent assay) and bactericidal activity were inhibited by de-N-acetylated MBPS and re-N-acetylated MBPS, which indicate that N-propionyl groups are not obligatory determinants for binding. The results of affinity selection from a preparation of N-Pr MBPS and matrix-assisted laser desorption ionization-time of flight mass spectroscopic analysis indicated that the minimal %%%epitope%%% recognized by the MAb is a MBPS disaccharide containing one de-N-acetylated residue. Thus, the bacterial capsular %%%epitope%%% recognized by this bactericidal, nonautoreactive, anti-group-B MAb likely contains de-N-acetyl residues.

8/7/2
DIALOG(R)File 5:Biosis Previews(R)

0015301008 BIOSIS NO.: 200500204810
Codon optimization of the BirA %%%enzyme%%% gene leads to higher expression and an improved efficiency of biotinylation of target proteins in mammalian cells
AUTHOR: Mechold Undine; Gilbert Cristele; Ogryzko Vasily (Reprint)
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JOURNAL: Journal of Biotechnology 116 (3): p245-249 March 30, 2005 2005
MEDIUM: print
ISSN: 0168-1656 (ISSN print)
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Biotinylation of proteins is an attractive alternative to '%%%epitope%%%-tagging', due to the strong biotin-(strept)avidin interaction and to the wide commercial availability of reagents for detection and purification of biotinylated macromolecules. Enzymatic biotinylation of target proteins in vivo using short biotin acceptor domains was described previously. Their use in mammalian cell requires expression of the bacterial biotinylation %%%enzyme%%% BirA. Here we describe the construction of a humanized version of BirA, with most of

the rare codons %%%replaced%%% by codons that are more frequently used in %%%human%%% cells. The humanized BirA is expressed better in mammalian cells, resulting in improved efficiency of biotinylation in vivo. We anticipate that the humanized BirA gene will find use in many applications that involve in vivo biotinylation.

8/7/3
DIALOG(R)File 5:Biosis Previews(R)

0015013863 BIOSIS NO.: 200400384652
Domain swapping in N-truncated %%%human%%% cystatin C
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JOURNAL: Journal of Molecular Biology 341 (1): p151-160 July 30, 2004 2004
MEDIUM: print
ISSN: 0022-2836 _(ISSN print)
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: %%%Human%%% cystatin C (HCC) inhibits papain-like cysteine proteases by a binding %%%epitope%%% composed of two beta-hairpin loops and the N-terminal segment. HCC is found in all body fluids and is present at a particularly high level in the cerebrospinal fluid. Oligomerization of HCC leads to amyloid deposits in brain arteries at advanced age but this pathological process is greatly accelerated with a naturally occurring Leu68Gln variant, resulting in fatal amyloidosis in early adult life. When proteins are extracted from %%%human%%% cystatin C amyloid deposits, an N-terminally truncated cystatin C (THCC) is found, lacking the first ten amino acid residues of the native sequence. It has been shown that the cerebrospinal fluid may cause this N-terminal truncation, possibly because of disintegration of the leucocytes normally present in this fluid, and the release of leucocyte proteolytic enzymes. HCC is the first disease-causing amyloidogenic protein for which oligomerization via 3D domain swapping has been observed. The aggregates arise in the crystallization buffer and have the form of 2-fold symmetric dimers in which a long alpha-helix of one molecule, flanked by two adjacent beta-strands, has %%%replaced%%% an identical domain of the other molecule, and vice versa. Consistent with a conformational change at one of the beta-hairpin loops of the binding %%%epitope%%%, the dimers (and also any other oligomers, including amyloid aggregates) are inactive as papain inhibitors. Here, we report the structure of N-truncated HCC, the dominant form of cystatin C in amyloid deposits. Although the protein crystallized under conditions that are drastically different from those for the full-length protein, the structure reveals dimerization by the same act of domain swapping. However, the new crystal structure is composed of four independent HCC dimers, none of which has the exact 2-fold symmetry of the full-length dimer. While the four dimers have the same overall topology, the exact relation between the individual domains shows a variability that reflects the flexibility at the dimer-specific open interface, which in the case of 3D domain-swapped HCC consists of beta-interactions between the open hinge loops and results in an unusually long intermolecular beta-sheet. The dimers are engaged in further quaternary interactions resulting in spherical, closed octameric assemblies that are identical to that present in the crystal of the full-length protein. The octamers interact via hydrophobic patches formed on the surface of the domain-swapped dimers as well as by extending the dimer beta-sheet through intermolecular contacts.

8/7/4
DIALOG(R)File 5:Biosis Previews(R)

0014459954 BIOSIS NO.: 200300415616
Expression of enzymatically-active phospholipase Cgamma2 in E. coli.
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JOURNAL: Journal of Biochemistry and Molecular Biology 35 (5): p508-512 September 30, 2003 2003
MEDIUM: print
ISSN: 1225-8687
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Phospholipase C-gamma-2 (PLCgamma2) activation is a key signaling event for many cell functions. In order to delineate the pathways that lead to PLCgamma2 activation, we devised a quick method for obtaining sufficient PLCgamma2. We obtained the full-length cDNA for %%%human%%% PLCgamma2 and expressed it in E. coli using the expression vector pT5T. To enhance the protein expression, tandem AGG-AGG arginine codons at the amino acid positions 1204-1205 were %%%replaced%%% by CGG-CGG arginine codons. The protein expression was detected in a Western blot analysis by both anti-PLCgamma2 antibodies and the antibodies that are raised against the tripeptide %%%epitope%%% (Glu-Glu-Phe) tag that are genetically-engineered to its carboxyl terminal. Crude lysates that were prepared from bacteria that express PLCgamma2 were found to catalyze the hydrolysis of phosphatidylinositol 4,5 bisphosphate. Similar to previous reports on PLCgamma2 that is isolated from mammalian tissue, the recombinant %%%enzyme%%% was Ca2+ dependent with optimal activity at 1-10 uM Ca2+.

8/7/5
DIALOG(R)File 5:Biosis Previews(R)

0014311463 BIOSIS NO.: 200300280182
Immune tolerance after long-term %%%enzyme%%%-%%%replacement%%% therapy among patients who have mucopolysaccharidosis I.
AUTHOR: Kakavanos Revecca; Turner Chris T; Hopwood John J; Kakkis Emil D; Brooks Doug A (Reprint)
AUTHOR ADDRESS: Lysosomal Diseases Research Unit, Department of Chemical Pathology, Women's and Children's Hospital, North Adelaide, SA, 5006, Australia**Australia
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JOURNAL: Lancet (North American Edition) 361 (9369): p1608-1613 May 10, 2003 2003
MEDIUM: print
ISSN: 0099-5355 _(ISSN print)
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Background %%%Enzyme%%%-%%%replacement%%% therapy has been assessed as a treatment for patients who have mucopolysaccharidosis I (alpha-L-iduronidase deficiency). We aimed to investigate the humoral immune response to recombinant %%%human%%% alpha-L-iduronidase among these patients. Methods We characterised the antibody titres and specific linear sequence %%%epitope%%% reactivity of serum antibodies to alpha-L-iduronidase for ten patients with mucopolysaccharidosis I, at the start of treatment and after 6, 12, 26, 52, and 104 weeks. We compared the values for patients' samples with those for samples from normal %%%human%%% controls. Findings Before %%%enzyme%%%-%%%replacement%%% therapy, all patients had low serum antibody titres to recombinant %%%human%%% alpha-L-iduronidase that were within the control range. Five of the ten patients produced higher-than-normal titres of antibody to the %%%replacement%%% protein during the treatment course (serum antibody titres 130 000-500 000 and high-affinity %%%epitope%%% reactivity). However, by week 26, antibody reactivity was reduced, and by week 104 all patients had low antibody titres and only low-affinity %%%epitope%%% reactivity. Patients who had mucopolysaccharidosis I with antibody titres

within the normal range at 6-12 weeks did not subsequently develop immune responses. Interpretation After 2 years of treatment, patients who initially had an immune reaction developed immune tolerance to alpha-L-iduronidase. This finding has positive implications for long-term %%%enzyme%%%-%%%replacement%%% therapy in patients who have mucopolysaccharidosis I.

8/7/6
DIALOG(R)File 5:Biosis Previews(R)

0014155069 BIOSIS NO.: 200300113788
Histidine 271 has a functional role in pig alpha-1,3galactosyltransferase %%%enzyme%%% activity.
AUTHOR: Lazarus Brooke D; Milland Julie; Ramsland Paul A; Mouhtouris Effie; Sandrin Mauro S (Reprint)
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JOURNAL: Glycobiology 12 (12): p793-802 December 2002 2002
MEDIUM: print
ISSN: 0959-6658
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: alpha(1,3)Galactosyltransferase (GT) is a Golgi-localized %%%enzyme%%% that catalyzes the transfer of a terminal galactose to N-acetyllactosamine to create Galalpha(1,3)Gal. This glycosyltransferase has been studied extensively because the Galalpha(1,3)Gal %%%epitope%%% is involved in hyperacute rejection of pig-to-%%%human%%% xenotransplants. The original crystal structure of bovine GT defines the amino acids forming the catalytic pocket; however, those directly involved in the interaction with the donor nucleotide sugars were not characterized. Comparison of amino acid sequences of GT from several species with the %%%human%%% A and B transferases suggest that His271 of pig GT may be critical for recognition of the donor substrate, UDP-Gal. Using pig GT as the representative member of the GT family, we show that %%%replacement%%% of His271 with Ala, Leu, or Gly caused complete loss of function, in contrast to %%%replacement%%% with Arg, another basic charged residue, which did not alter the ability of GT to produce Galalpha(1,3)Gal. Molecular modeling showed that His271 may interact directly with the Gal moiety of UDP-Gal, an interaction possibly retained by replacing His with Arg. However, replacing His271 with amino acids found in alpha(1,3)GalNAc transferases did not change the donor nucleotide specificity. Thus His271 is critical for enzymatic function of pig GT.

8/7/7
DIALOG(R)File 5:Biosis Previews(R)

0014146056 BIOSIS NO.: 200300104775
%%%Epitope%%% analysis of antibodies in Japanese to %%%human%%% cytomegalovirus phosphoprotein 150 with synthetic peptides.
AUTHOR: Takahashi Isao (Reprint); Sugiura Sayoko; Ohta Hirotoshi; Ozawa Kazuo; Kamiya Tadashi
AUTHOR ADDRESS: Japanese Red Cross Aichi Blood Center, 539-3 Minami-yamaguchi, Seto, Aichi, 489-8555, Japan**Japan
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JOURNAL: Bioscience Biotechnology and Biochemistry 66 (11): p2402-2405 November 2002 2002
MEDIUM: print
ISSN: 0916-8451
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Serological detection of antibodies specific to %%%human%%% cytomegalovirus (HCMV) is not reliable because the assay uses the whole HCMV protein fraction. Antigenic materials composed of well-characterized viral proteins are being tried for serodiagnosis in Europe. Epitopes of antibodies to HCMV phosphoprotein 150 (pp150) encoded by UL32 in Japanese individuals were investigated for comparison with the results in Europe. The major epitopes on amino acid residues 496 to 652 of HCMV pp150 were identified and the detection of antibodies with an %%%enzyme%%%-linked immunosorbent assay (ELISA) of synthetic peptides against the main epitopes was established. Fifteen seropositive and five seronegative serum samples for the %%%epitope%%% mapping and 131 seropositive and 50 seronegative samples for ELISA were investigated. Overlapping 15-mer peptides moving by two amino acids through V496-H652 were synthesized. The main %%%epitope%%% regions were V508-D530, L526-Q544, S536-D554, T616-G634, S624-P642, and L632-H652. When each peptide was conjugated with bovine serum albumin for ELISA, 80.9% of the seropositive samples were judged to be positive. The results of this study are the same as those for European sera, so the antigenic materials developed in Europe might be used to %%%replace%%% the whole HCMV protein fraction in Japanese.

8/7/8
DIALOG(R)File 5:Biosis Previews(R)

0014091692 BIOSIS NO.: 200300049041
Surface display of foreign epitopes on the Lactobacillus brevis S-layer.
AUTHOR: Avall-Jaaskelainen Silja; Kyla-Nikkila Kari; Kahala Minna; Miikkulainen-Lahti Terhi; Palva Airi (Reprint)
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JOURNAL: Applied and Environmental Microbiology 68 (12): p5943-5951
December 2002 2002
MEDIUM: print
ISSN: 0099-2240 _(ISSN print)
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: So far, the inability to establish viable Lactobacillus surface layer (S-layer) null mutants has hampered the biotechnological applications of Lactobacillus S-layers. In this study, we demonstrate the utilization of Lactobacillus brevis S-layer subunits (SlpA) for the surface display of foreign antigenic epitopes. With an inducible expression system, L. brevis strains producing chimeric S-layers were obtained after testing of four insertion sites in the slpA gene for poliovirus %%%epitope%%% VP1, that comprises 10 amino acids. The %%%epitope%%% insertion site allowing the best surface expression was used for the construction of an integration vector carrying the gene region encoding the c-Myc epitopes from the %%%human%%% c-myc proto-oncogene, which is composed of 11 amino acids. A gene %%%replacement%%% system was optimized for L. brevis and used for the %%%replacement%%% of the wild-type slpA gene with the slpA-c-myc construct. A uniform S-layer, displaying on its surface the desired antigen in all of the S-layer protein subunits, was obtained. The success of the gene %%%replacement%%% and expression of the uniform SlpA-c-Myc recombinant S-layer was confirmed by PCR, Southern blotting MALDI-TOF mass spectrometry, whole-cell %%%enzyme%%%-linked immunosorbent assay, and immunofluorescence microscopy. Furthermore, the integrity of the recombinant S-layer was studied by electron microscopy, which indicated that the S-layer lattice structure was not affected by the presence of c-Myc epitopes. To our knowledge, this is the first successful expression of foreign epitopes in every S-layer subunit of a Lactobacillus S-layer while still maintaining the S-layer lattice structure.

8/7/9
DIALOG(R)File 5:Biosis Previews(R)

0013822357 BIOSIS NO.: 200200415868
Recombinant %%%human%%% adenovirus: Targeting to the %%%human%%% transferrin receptor improves gene transfer to brain microcapillary endothelium
AUTHOR: Xia Haibin; Anderson Brian; Mao Qinwen; Davidson Beverly L (Reprint)
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JOURNAL: Journal of Virology 74 (23): p11359-11366 December, 2000 2000
MEDIUM: print
ISSN: 0022-538X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Some inborn errors of metabolism due to deficiencies of soluble lysosomal enzymes cause global neurodegenerative disease. Representative examples include the infantile and late infantile forms of the ceroid lipofuscinoses (CLN1 or CLN2 deficiency, respectively) and mucopolysaccharidoses type VII (MPS VII), a deficiency of beta-glucuronidase. Treatment of the central nervous system component of these disorders will require widespread protein or %%%enzyme%%% %%%replacement%%%, either through dissemination of the protein or through dissemination of a gene encoding it. We hypothesize that transduction of brain microcapillary endothelium (BME) with recombinant viral vectors, with secretion of %%%enzyme%%% product basolaterally, could allow for widespread %%%enzyme%%% dissemination. To achieve this, viruses should be modified to target the BME. This requires (i) identification of a BME-resident target receptor, (ii) identification of motifs targeted to that molecule, (iii) the construction of modified viruses to allow for binding to the target receptor, and (iv) demonstrated transduction of receptor-expressing cells. In proof of principal experiments, we chose the %%%human%%% transferrin receptor (hTfR), a molecule found at high density on %%%human%%% BME. A nonamer phage display library was panned for motifs which could bind hTfR. Forty-three clones were sequenced, most of which contained an AKxxK/R, KxKxPK/R, or KxK motif. Ten peptides representative of the three motifs were cloned into the HI loop of adenovirus type 5 fiber. All motifs tested retained their ability to trimerize and bind transferrin receptor, and seven allowed for recombinant adenovirus production. Importantly, the fiber-modified viruses facilitated increased gene transfer (2- to 34-fold) to hTfR expressing cell lines and %%%human%%% brain microcapillary endothelia expressing high levels of endogenous receptor. Our data indicate that adenoviruses can be modified in the HI loop for expanded tropism to the hTfR.

8/7/10
DIALOG(R)File 5:Biosis Previews(R)

0013606131 BIOSIS NO.: 200200199642
HPA-1a phenotype-genotype discrepancy reveals a naturally occurring Arg93Gln substitution in the platelet beta3 integrin that disrupts the HPA-1a %%%epitope%%%
AUTHOR: Watkins Nicholas A; Schaffner-Reckinger Elisabeth; Allen David L; Howkins Graham J; Brons Nicolaas H C; Smith Graham A; Metcalfe Paul; Murphy Michael F; Kieffer Nelly; Ouwehand Willem H (Reprint)
AUTHOR ADDRESS: Dept of Haematology, Division of Transfusion Medicine, University of Cambridge, Cambridge, CB2 2PT, UK**UK
JOURNAL: Blood 99 (5): p1833-1839 March 1, 2002 2002
MEDIUM: print
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A single nucleotide polymorphism (SNP) at position 196 in the beta3 integrin causes a Leu33Pro substitution in the mature protein. Alloimmunization against the beta3Leu33 form (%%%human%%% platelet

antigen (HPA)-1a, PIA1, Zwa) in patients who are beta3Pro33 homozygous (HPA-1b1b, PIA2A2, Zwbb) causes neonatal alloimmune thrombocytopenia, posttransfusion purpura, or refractoriness to platelet transfusion. Studies with recombinant proteins have demonstrated that amino acids 1 to 66 and 288 to 490 of the beta3 integrin contribute to HPA-1a %%%epitope%%% formation. In determining the HPA-1a status of more than 6000 donors, we identified a donor with an HPA-1aweak phenotype and an HPA-1a1b genotype. The platelets from this donor had normal levels of surface alphaIIbbeta3 but reacted only weakly with monoclonal and polyclonal anti-HPA-1a by whole blood %%%enzyme%%%-linked immunosorbent assay (ELISA), flow cytometry, and sandwich ELISA. We reasoned that an alteration in the primary nucleotide sequence of the beta3Leu33 allele of this donor was disrupting the HPA-1a %%%epitope%%%. In agreement with this hypothesis, sequencing platelet RNA-derived alphaIIb and beta3 cDNA identified a novel G/A SNP at position 376 of the beta3 integrin that encodes for an Arg93Gln %%%replacement%%% in the beta3Leu33 allele. Coexpression of the beta3Leu33Gln93 encoding cDNA in Chinese hamster ovary cells with %%%human%%% alphaIIb cDNA showed that the surface-expressed alphaIIbbeta3 reacted normally with beta3 integrin-specific monoclonal antibodies but only weakly with monoclonal anti-HPA-1a. Our results show that an Arg93Gln mutation in the beta3Leu33 encoding allele disrupts the HPA-1a %%%epitope%%%, suggesting that Arg93 contributes to the formation of the HPA-1a B-cell %%%epitope%%%.

8/7/11
DIALOG(R)File 5:Biosis Previews(R)

0013270102 BIOSIS NO.: 200100441941
The GOR gene product cannot cross-react with hepatitis C virus in humans
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JOURNAL: Clinical and Experimental Immunology 124 (3): p429-434 June, 2001 2001
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LANGUAGE: English

ABSTRACT: GOR (GOR47-1) is an %%%epitope%%% thought to be a host-derived antigen cross-reactive with hepatitis C virus (HCV) since it was isolated from a cDNA library of host animals reactive with sera of HCV-positive patients. An %%%enzyme%%% immunosorbent assay (ELISA) using this %%%epitope%%% as antigen is of sufficient sensitivity and specificity for screening patients with HCV. However, the relationship between GOR47-1 %%%epitope%%% and autoimmune phenomena associated with HCV infection or autoimmune hepatitis is controversial. Here we isolated the %%%human%%% GOR gene and found that the GOR47-1 %%%epitope%%% was not translated in humans due to a single base %%%replacement%%% from chimpanzee. Furthermore, we found some patients who had antibodies against another %%%epitope%%%, which is translated (GOR1-125) in humans, although there was no correlation between the existence of anti-GOR47-1 or anti-GOR1-125 Ab and autoimmune phenomena. Serum IgG levels did not influence the titres of these antibodies. Taken together with the results of several other studies, our finding that the GOR47-1 %%%epitope%%% cannot be translated into a protein suggests that there is little relationship between autoimmunity and the GOR gene product in %%%human%%% beings. We also discuss here the possible mechanism of cross-reactivity between HCV and the GOR gene product.

8/7/12
DIALOG(R)File 5:Biosis Previews(R)

0013199660 BIOSIS NO.: 200100371499
N-glycan structure of a short-lived variant of ribophorin I expressed in the MadIA214 glycosylation-defective cell line reveals the role of a

mannosidase that is not ER mannosidase I in the process of glycoprotein degradation
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JOURNAL: Glycobiology 11 (7): p565-576 July, 2001 2001
MEDIUM: print
ISSN: 0959-6658
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LANGUAGE: English

ABSTRACT: A soluble form of ribophorin I (RI332) is rapidly degraded in Hela and Chinese hamster ovary (CHO) cells by a cytosolic proteasomal pathway, and the N-linked glycan present on the protein may play an important role in this process. Specifically, it has been suggested that endoplasmic reticulum (ER) mannosidase I could trigger the targeting of improperly folded glycoproteins to degradation. We used a CHO-derived glycosylation-defective cell line, MadIA214, for investigating the role of mannosidase(s) as a signal for glycoprotein degradation. Glycoproteins in MadIA214 cells carry truncated Glc1Man5GlcNAc2 N-glycans. This oligomannoside structure interferes with protein maturation and folding, leading to an alteration of the ER morphology and the detection of high levels of soluble oligomannoside species caused by glycoprotein degradation. An HA-%%%epitope%%%-tagged soluble variant of ribophorin I (RI332-3HA) expressed in MadIA214 cells was rapidly degraded, comparable to control cells with the complete Glc3Man9GlcNAc2 N-glycan. ER-associated degradation (ERAD) of RI332-3HA was also proteasome-mediated in MadIA214 cells, as demonstrated by inhibition of RI332-3HA degradation with agents specifically blocking proteasomal activities. Two inhibitors of alpha1,2-mannosidase activity also stabilized RI332-3HA in the glycosylation-defective cell line. This is striking, because the major mannosidase activity in the ER is the one of mannosidase I, specific for a mannose alpha1,2-linkage that is absent from the truncated Man5 structure. Interestingly, though the Man5 derivative was present in large amounts in the total protein pool, the two major species linked to RI332-3HA shortly after synthesis consisted of Glc1Man5 and Man4, being %%%replaced%%% by Man4 and Man3 when proteasomal degradation was inhibited. In contrast, the untrimmed intermediate of RI332-3HA was detected in mutant cells treated with mannosidase inhibitors. Our results unambiguously demonstrate that an alpha1,2-mannosidase that is not ER mannosidase I is involved in ERAD of RI332-3HA in the glycosylation-defective cell line, MadIA214.

8/7/13
DIALOG(R)File 5:Biosis Previews(R)

0012923602 BIOSIS NO.: 200100095441
Recombinant mono and poly antigens to detect cytomegalovirus-specific IgM in %%%human%%% sera by %%%enzyme%%% immunoassay
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JOURNAL: Official Gazette of the United States Patent and Trademark Office Patents 1235 (2): June 13, 2000 2000
MEDIUM: e-file
ISSN: 0098-1133
DOCUMENT TYPE: Patent
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A mixture of recombinant mono- and poly-%%%epitope%%% proteic materials able to fully %%%replace%%% the viral antigens when used in an %%%enzyme%%% immunoassay (EIA) is disclosed; the mixture includes a poly-%%%epitope%%% fusion protein having a first region formed by an amino acid sequence (H10) corresponding to that of the last 233 amino acids of the COOH terminus of the viral protein p52 or to a part thereof, a second region formed by an amino acid sequence (F3) corresponding to that of the

last 43 amino acids of the COOH terminus of viral protein pp150 or to a part thereof, and a third region formed by an amino acid sequence (A1C2) corresponding to that taken from aa 595 to aa 614, proceeding in direction 5'fwdarw3', of the same viral protein pp150; and, in combination, a second fusion protein including a sequence of amino acids corresponding to that taken, proceeding in direction 5'fwdarw3', from aa 297 to aa 510 of the viral major matrix protein pp65 encoded by the viral gene UL83 and a third fusion protein including a sequence of amino acids corresponding to that taken, proceeding in direction 5'fwdarw3', from aa 117 to aa 373 of the viral assembly protein pp38 encoded by the viral gene UL80a. These three fusion proteins may be used combined together for the preparation of an ELISA test kit for detection of Cytomegalovirus-specific IgM in %%%human%%% sera.

8/7/14
DIALOG(R)File 5:Biosis Previews(R)

0012923159 BIOSIS NO.: 200100094998
Identifying diagnostic peptides for Lyme disease through %%%epitope%%% discovery
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JOURNAL: Clinical and Diagnostic Laboratory Immunology 8 (1): p150-160 January, 2001 2001
MEDIUM: print
ISSN: 1071-412X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Serum antibodies from patients with Lyme disease (LD) were used to affinity select peptide epitopes from 12 large random peptide libraries in phage display format. The selected peptides were surveyed for reactivity with a panel of positive sera (from LD patients) and negative sera (from subjects without LD), thus identifying 17 peptides with a diagnostically useful binding pattern: reactivity with at least three positive sera and no reactivity with any of the negative sera. The peptides define eight sequence motifs, none of which can be matched convincingly with segments of proteins from Borrelia burgdorferi, the LD pathogen; evidently, then, they are "mimotopes," mimicking natural pathogen epitopes without matching contiguous amino acids of pathogen proteins. Peptides like these could be the basis of a new diagnostic %%%enzyme%%%-linked immunosorbent assay for LD, with sufficient specificity and sensitivity to %%%replace%%% expensive immunoblotting tests that are currently required for definitive serological diagnosis. Moreover, the method used to discover these peptides did not require any knowledge of the pathogen and involved generic procedures that are applicable to almost any infectious disease, including emerging diseases for which no pathogen has yet been identified.

8/7/15
DIALOG(R)File 5:Biosis Previews(R)

0012699824 BIOSIS NO.: 200000418137
In vivo functional analysis of the %%%human%%% mitochondrial DNA polymerase POLG expressed in cultured %%%human%%% cells
AUTHOR: Spelbrink Johannes N; Toivonen Janne M; Hakkaart Gerrit A J; Kurkela Johanna M; Cooper Helen M; Lehtinen Sanna K; Lecrenier Nicolas; Back Jaap Willem; Speijer Dave; Foury Francoise; Jacobs Howard T (Reprint)
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JOURNAL: Journal of Biological Chemistry 275 (32): p24818-24828 August 11, 2000 2000
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LANGUAGE: English

ABSTRACT: The %%%human%%% gene POLG encodes the catalytic subunit of mitochondrial DNA polymerase, but its precise roles in mtDNA metabolism in vivo have not hitherto been documented. By expressing POLG fusion proteins in cultured %%%human%%% cells, we show that the %%%enzyme%%% is targeted to mitochondria, where the Myc %%%epitope%%%-tagged POLG is catalytically active as a DNA polymerase. Long-term culture of cells expressing wild-type POLG-myc revealed no alterations in mitochondrial function. Expression of POLG-myc mutants created dominant phenotypes demonstrating important roles for the protein in mtDNA maintenance and integrity. The D198A amino acid %%%replacement%%% abolished detectable 3'-5' (proofreading) exonuclease activity and led to the accumulation of a significant load (1:1700) of mtDNA point mutations during 3 months of continuous culture. Further culture resulted in the selection of cells with an inactivated mutator polymerase, and a reduced mutation load in mtDNA. Transient expression of POLG-myc variants D890N or D1135A inhibited endogenous mitochondrial DNA polymerase activity and caused mtDNA depletion. Deletion of the POLG CAG repeat did not affect enzymatic properties, but modestly up-regulated expression. These findings demonstrate that POLG exonuclease and polymerase functions are essential for faithful mtDNA maintenance in vivo, and indicate the importance of key residues for these activities.

8/7/16
DIALOG(R)File 5:Biosis Previews(R)

0012527658 BIOSIS NO.: 200000245971
Serum crosslaps for monitoring the response in individuals undergoing antiresorptive therapy
AUTHOR: Christgau S (Reprint); Bitsch-Jensen O; Bjarnason N Hanover; Henriksen E Gamwell; Qvist P; Alexandersen P; Henriksen D Bang
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JOURNAL: Bone (New York) 26 (5): p505-511 May, 2000 2000
MEDIUM: print
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LANGUAGE: English

ABSTRACT: The Serum CrossLaps (CTx) %%%enzyme%%%-linked immunosorbent assay (ELISA) is specific for a cross-linked, beta-aspartateisomerized form of the %%%epitope%%% EKAHDGGR derived from the carboxyterminal telopeptide region of type I collagen alpha1 chain. Collagen type I fragments reactive in the CTx assay are released during osteoclastic bone resorption and can be used as a measure of bone resorption activity. Our objectives were to assess the intraindividual variation of serum CTx concentration as well as the clinical value of the serum CTx assay for monitoring antiresorptive therapy in individual patients. The influence of the sampling time and fasting on the serum CTx measurements was studied with the aim of determining an optimal sampling protocol. Studies of circadian variation in serum CTx concentration in 15 postmenopausal women showed that fasting significantly reduced the average circadian variation of the marker from 36% to 8.7%. This was further supported by assessing short-term (2 weeks) intraindividual variation in ten postmenopausal women who were sampled in the morning, either fasting or nonfasting. The average short-term intraindividual coefficient of variation (CV) was 7.9% in the samples obtained from fasting women, and 14.3% in the samples obtained from nonfasting women. The long-term intraindividual biological variation was 13.4% in 44 postmenopausal women sampled every 6 months (fasting morning samples) over a 1 year period. The ability of the serum CTx assay to monitor individual responses to antiresorptive therapy was assessed in studies of the effects of hormone %%%replacement%%% therapy (HRT) and bisphosphonate (alendronate). Serum samples (morning fasting) were obtained from postmenopausal women treated with either bisphosphonate or HRT at baseline and then after various

timepoints of therapy. Spine bone mineral density (BMD) measurements were carried out and the annual percentage change in spine BMD (alphaBMD) was calculated. Sixteen of 17 (94%) of the HRT-treated and 12 of 13 (92%) of the bisphosphonate-treated women showed a decrease in serum CTx after 6 months that was greater than the calculated least significant change (LSC) of the marker (LSCCTx). In contrast, only 59% of the HRT-treated and 64% of the bisphosphonate-treated women showed a response in spine BMD greater than the LSCBMD after 1 year follow-up. When the two study populations were combined, the decrease in serum CTx after 6 months showed an analytical sensitivity of 83.8% and specificity of 100% to differentiate women with a gain in spine BMD (alphaBMD) > 0%) from women with a loss in spine BMD (alphaBMD < 0%). In conclusion, the serum CTx showed high specificity and sensitivity for monitoring individual responses to antiresorptive therapy. More than 92% of the treated women showed significant responses in serum CTx measurements after 6 months.

8/7/17
DIALOG(R)File 5:Biosis Previews(R)

0012297029 BIOSIS NO.: 200000015342
Immunoglobulin variable genes and %%%epitope%%% recognition of %%%human%%% monoclonal anti-Ro 52-kd in primary Sjogren's syndrome
AUTHOR: Elagib Kamal E E (Reprint); Tengner Pia; Levi Michael; Jonsson Roland; Thompson Keith M; Natvig Jacob B; Wahren-Herlenius Marie
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JOURNAL: Arthritis and Rheumatism 42 (11): p2471-2481 Nov., 1999 1999
MEDIUM: print
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Objective: To clone and characterize %%%human%%% anti-Ro/SSA autoantibodies from a patient with primary Sjogren's syndrome (pSS). Methods: Monoclonal antibodies (mAb) were raised from the peripheral blood of a patient with pSS using Epstein-Barr virus transformation and a hybridoma technique. Specificity was determined using cell extracts, recombinant Ro 52-kd, Ro 60-kd, and La proteins as well as Ro 52-kd peptides in %%%enzyme%%%-linked immunosorbent assay (ELISA) and Western blot. The immunofluorescence pattern was analyzed using cultured %%%human%%% and mouse cell lines. Complementary DNA was amplified by polymerase chain reaction, and Ig variable (V)-region genes were directly sequenced. Results: Two %%%human%%% anti-Ro 52-kd mAb of IgM isotype, denoted SG1 and SG3, were cloned from the peripheral blood of a patient with pSS. The 2 mAb reacted with the Ro 52-kd antigen in cell extracts of %%%human%%% cell lines and mouse cell lines, and with purified %%%human%%% recombinant Ro 52-kd protein in ELISA and Western blot. SG1 reacted specifically with 1 peptide, amino acids 136-156, of the Ro 52-kd protein, and SG3 was mapped to react with a recombinant fragment representing amino acids 136-292. Immunofluorescence studies revealed cytoplasmic staining with both mAb. Both were encoded by VH3-family genes. SG1 was highly homologous to the DP-77 germ-line gene, with 2 %%%replacement%%% mutations and 1 silent. It utilized the DPL-11 germ-line gene from the Vlambda2-family gene, with 1 silent mutation. SG3 was 100% homologous to the DP-47 germ-line gene, combined with a Vkappa1-family gene that was 100% homologous to the A30 germ-line gene. Conclusion: Two %%%human%%% mAb were demonstrated to be specific for the Ro 52-kd protein and to be directed against 2 different epitopes, 1 linear and 1 conformation-dependent, within a region previously described to be immunodominant. Somatic hypermutation appeared to be of minor importance in generating these 2 specificities.

8/7/18
DIALOG(R)File 5:Biosis Previews(R)

0012189817 BIOSIS NO.: 199900449477

The role of amino acids in extracellular loops of the %%%human%%% P2Y1 receptor in surface expression and activation processes
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JOURNAL: Journal of Biological Chemistry 274 (21): p14639-14647 May 21, 1999 1999
MEDIUM: print
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LANGUAGE: English

ABSTRACT: The P2Y1 receptor is a membrane-bound G protein-coupled receptor stimulated by adenine nucleotides. Using alanine scanning mutagenesis, the role in receptor activation of charged amino acids (Asp, Glu, Lys, and Arg) and cysteines in the extracellular loops (EL) of the %%%human%%% P2Y1 receptor has been investigated. The mutant receptors were expressed in COS-7 cells and measured for stimulation of phospholipase C induced by the potent agonist 2-methylthioadenosine-5'-diphosphate (2-MeSADP). In addition to single point mutations, all receptors carried the hemagglutinin %%%epitope%%% at the N-terminus for detection of cell-surface expression. The C124A and C202A mutations, located near the exofacial end of transmembrane helix 3 and in EL2, respectively, ablated phospholipase C stimulation by ltoreq100 muM 2-MeSADP. Surface %%%enzyme%%%-linked immunosorbent assay detection of both mutant receptors showed <10% expression, suggesting that a critical disulfide bridge between EL2 and the upper part of transmembrane 3, as found in many other G protein-coupled receptors, is required for proper trafficking of the P2Y1 receptor to the cell surface. In contrast, the C42A and C296A mutant receptors (located in the N-terminal domain and EL3) were activated by 2-MeSADP, but the EC50 values were >1000-fold greater than for the wild-type receptor. The double mutant receptor C42A/C296A exhibited no additive shift in the concentration-response curve for 2-MeSADP. These data suggest that Cys42 and Cys296 form another disulfide bridge in the extracellular region, which is critical for activation. %%%Replacement%%% of charged amino acids produced only minor changes in receptor activation, with two remarkable exceptions. The E209A mutant receptor (EL2) exhibited a >1000-fold shift in EC50. However, if Glu209 were substituted with amino acids capable of hydrogen bonding (Asp, Gln, or Arg), the mutant receptors responded like the wild-type receptor. Arg287 in EL3 was impaired similarly to Glu209 when substituted by alanine. Substitution of Arg287 by lysine, another positively charged residue, failed to fully restore wild-type activity.

8/7/19
DIALOG(R)File 5:Biosis Previews(R)

0012183976 BIOSIS NO.: 199900443636
Fibrinogen cleavage by the Streptococcus pyogenes extracellular cysteine protease and generation of antibodies that inhibit %%%enzyme%%% proteolytic activity
AUTHOR: Matsuka Yury V (Reprint); Pillai Subramonia; Gubba Siddeswar; Musser James M; Olmsted Stephen B
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JOURNAL: Infection and Immunity 67 (9): p4326-4333 Sept., 1999 1999
MEDIUM: print
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ABSTRACT: The extracellular cysteine protease from Streptococcus pyogenes is a virulence factor that plays a significant role in host-pathogen interaction. Streptococcal protease is expressed as an inactive 40-kDa precursor that is autocatalytically converted into a 28-kDa mature

(active) %%%enzyme%%%. %%%Replacement%%% of the single cysteine residue involved in formation of the %%%enzyme%%% active site with serine (C192S mutation) abolished detectable proteolytic activity and eliminated autocatalytic processing of zymogen to the mature form. In the present study, we investigated activity of the wild-type (wt) streptococcal protease toward %%%human%%% fibrinogen and bovine casein. The former is involved in blood coagulation, wound healing, and other aspects of hemostasis. Treatment with streptococcal protease resulted in degradation of the COOH-terminal region of fibrinogen alpha chain, indicating that fibrinogen may serve as an important substrate for this %%%enzyme%%% during the course of %%%human%%% infection. Polyclonal antibodies generated against recombinant 40- and 28-kDa (r40- and r28-kDa) forms of the C192S streptococcal protease mutant exhibited high %%%enzyme%%% -linked immunosorbent assay titers but demonstrated different inhibition activities toward proteolytic action of the wt %%%enzyme%%%. Activity of the wt protease was readily inhibited when the reaction was carried out in the presence of antibodies generated against r28-kDa C192S mutant. Antibodies produced against r40-kDa C192S mutant had no significant effect on proteolysis. These data suggest that the presence of the NH2-terminal prosegment prevents generation of functionally active antibodies and indicate that inhibition activity of antibodies most likely depends on their ability to bind the active-site region %%%epitope%%%(s) of the protein.

8/7/20
DIALOG(R)File 5:Biosis Previews(R)

0012109256 BIOSIS NO.: 199900368916
Immune response to %%%enzyme%%% %%%replacement%%% therapy: 4-sulfatase %%%epitope%%% reactivity of plasma antibodies from MPS VI cats
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JOURNAL: Molecular Genetics and Metabolism 67 (3): p194-205 July, 1999
1999
MEDIUM: print
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LANGUAGE: English

ABSTRACT: The mucopolysaccharidoses (MPS) are a group of multiple pathology disorders which are part of a larger group of genetic diseases known as lysosomal storage disorders. %%%Enzyme%%% %%%replacement%%% therapy (ERT) has been developed as a therapy for MPS patients. However, immune responses to ERT have been reported in MPS animal models and in %%%human%%% Gaucher patients. Antibodies can have adverse effects during ERT, which include hypersensitivity/anaphylactic reactions, %%%enzyme%%% inactivation, and %%%enzyme%%% degradation. This study aimed to characterize the immune response to ERT in a feline model of MPS VI, by defining the %%%epitope%%% reactivity of cat plasma antibody against %%%human%%% recombinant N-acetylgalactosamine 4-sulfatase (4-sulfatase) %%%replacement%%% protein. For MPS VI cat plasma, antibody reactivity was observed prior to ERT, with distinct regions of 4-sulfatase linear sequence displaying low affinity antibody reactivity. There was an increase in antibody titer to 4-sulfatase for MPS VI cats post-ERT, with the majority of the immune response detected to linear sequence epitopes. One cat displayed a high titer and high affinity %%%epitope%%% reactivity following prolonged exposure (gtoreq9 months) to the %%%replacement%%% protein. MPS VI cats on shorter term ERT (3 months) showed high titers to 4-sulfatase and similar patterns of %%%epitope%%% reactivity, but lower affinity antibody reactivity, when compared to the latter cat. This study reports the linear amino acid sequence reactivity and nature of the immune response produced to 4-sulfatase before and after ERT. The monitoring of antibody production during %%%replacement%%% therapy is an important consideration for patient management, as high titer antibodies can affect the efficacy of therapy.

8/7/21
DIALOG(R)File 5:Biosis Previews(R)

0012052464 BIOSIS NO.: 199900312124
Analysis of the C5a anaphylatoxin core domain using a C5a phage library selected on differentiated U937 cells
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JOURNAL: Molecular Immunology 36 (2): p145-152 Feb., 1999 1999
MEDIUM: print
ISSN: 0161-5890
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ABSTRACT: The %%%human%%% anaphylatoxin C5a is a 74-amino acid comprising polypeptide with a plethora of biological functions. Site directed mutagenesis studies suggest that several residues within the core and the C-terminus mediate the interaction with the C5a receptor. However, the contribution of particular core residues to receptor binding remained to be clarified. By means of the phage display technique, the loop between positions 35-40 was randomly mutated and the resulting C5a(35-40) fusion phage library affinity selected on C5a receptor expressing U937 cells. After five rounds of affinity enrichment, residues Arg37 and Arg40 were preferably selected. Enrichment was as high as 100% for Arg37 and 79% for Arg40. No significant enrichment of consensus residues could be obtained for positions 35, 36, 38 and 39. The core mutant C5a(A35E36R37A38S39R40), in which only Arg37/40 and Ala38 are of the native C5a sequence, was as potent as native C5a in both receptor binding and %%%enzyme%%% release examined on U937 cells. In contrast, %%%replacement%%% of Arg40 as in the mutant C5a(Q35E36R37I38L39N40) resulted in a 10-fold decrease in both binding and functional activities. Thus, selected out of a multiplicity of possibilities by the natural binding partner, Arg37 as well as Arg40 appear to be anchor residues in binding to the C5a receptor.

8/7/22
DIALOG(R)File 5:Biosis Previews(R)

0011791745 BIOSIS NO.: 199900051405
Molecular fine-specificity analysis of antibody responses to %%%human%%% cytomegalovirus and design of novel synthetic-peptide-based serodiagnostic assays
AUTHOR: Greijer Astrid E (Reprint); Van De Crommert Jos M G; Stevens Servi J C; Middeldorp Jaap M
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JOURNAL: Journal of Clinical Microbiology 37 (1): p179-188 Jan., 1999 1999
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LANGUAGE: English

ABSTRACT: To identify single immunodominant marker proteins which can %%%replace%%% complex virion antigen in serodiagnostic assays, we investigated in detail the molecular fine specificity of antibody responses in different individuals with latent or active %%%human%%% cytomegalovirus (HCMV) infection. An overview of the HCMV proteins recognized by %%%human%%% antibodies was obtained by immunoblotting. For selected immunodominant proteins the %%%epitope%%% fine specificity of the antibody response was determined by a peptide-scanning %%%enzyme%%% -linked immunosorbent assay (ELISA). %%%Epitope%%% clusters were synthesized as combination peptides and were used for further serologic analysis of immunoglobulin M (IgM) and IgG reactivities with panels of sera from different groups of patients in comparison to those with

cytomegalovirus (CMV) virion antigen. Several serum samples had significantly higher reactivities with peptides than with the CMV virion antigen. However, individual serum samples occasionally recognized diverse peptide epitopes, stressing the importance of using combinations of peptides in serologic assays. From these studies we were able to define a specific combination of peptides derived from pp52 (UL44) and pp150 (UL32) for the specific and highly sensitive early detection of HCMV IgM, whereas a combination of peptides from pp150 (UL32), gB (UL55), and pp28 (UL99) was selected to give optimal and specific reactivity with HCMV IgG. On the basis of the results obtained with these peptide combinations, new, highly specific serodiagnostic assays were constructed. These assays had sensitivities of 98.9 and 96.4% for IgG and IgM, respectively, in comparison with the results obtained with the "gold standard," the virion antigen-based ELISA. From the results of this study we conclude that specific combinations of highly defined synthetic peptides can %%%replace%%% complex HCMV virion extracts used in current serodiagnostics and may add to further standardization of HCMV serology.

8/7/23
DIALOG(R)File 5:Biosis Previews(R)

0011785826 BIOSIS NO.: 199900045486
Novel monoclonal antibodies to putative selectin carbohydrate ligands that inhibit selectin binding to myeloid cells
AUTHOR: Zhang Ke; Chuluyan H E; Hardie D; Shen D-C; Larsen Robert; Issekutz Andrew (Reprint)
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Four newly developed monoclonal antibodies (MAbs) are characterized using flowcytometry, %%%enzyme%%%-linked immunoadsorbent assay (ELISA), immunoprecipitation and Western blots, carbohydrate %%%epitope%%% mapping, glycosidase cleavage, and competition binding assays. Their effects on selectin binding to myeloid cells was tested. These MAbs react only with myeloid cells. MAbs CI-1, BU60, and HIM95 recognize epitopes expressed by CD11/CD18 (beta2) integrins, while HI247 and CSLEX1 do not. The epitopes require Lewis x (Galbeta1-4 (Fucalpha1-3)GlcNAc) based on reactivity with oligosaccharide-polyacrylamide-biotin or oligosaccharide-BSA conjugates. MAb HI247 recognizes a related structure, sialyl-Lewis x, NeuAcalpha2-3GaLbeta1-4(Fucalpha1-3)GlcNAc. The three MAbs against Lewis X show some minor differences in their reactivity such as recognizing their antigens on CD11/CD18 integrins after endo-beta-galactosidase treatment and recognizing free Lewis x. The hydroxyl group on C-3 of the terminal galactose is important for recognition by MAb CI-1, BU60, and HIM95 as its substitution with sulfo group of sialic acid abolishes the binding of these MAbs. The C-3 sialic acid is crucial for the binding of MAb HI247. Its %%%replacement%%% by sulphate or its cleavage by sialidase eliminates recognition by this MAb. MAbs HI247 and CSLEX-1 did not react in ELISA with immobilized CD11/CD18, suggesting that the majority of sialyl Lewis x on CD11/CD18 molecules may have sialic acid 6-linked rather than 3-linked to galactose. Unexpectedly, MAb BU60 inhibited binding of P-selectin mu chain chimera to HL-60 or U937 cells, while CI-1, HIM95 and three other defined anti-Lewis x MAbs (6C7, M6-1 and LeuM1) did not. MAb HI247 inhibited binding of both E- and P-selectin chimeras to these cell lines more effectively than several characterized MAbs (CSLEX-1, FH6, HECA-452) to sialyl Lewis x and related oligosaccharides. Certain combinations of these anticarbohydrate MAbs had additive inhibitory effects on selectin binding, suggesting a potential application of these new MAbs in cell adhesion/migration and tumor metastasis studies.

8/7/24
DIALOG(R)File 5:Biosis Previews(R)

0011510810 BIOSIS NO.: 199800305057
Mapping and serodiagnostic application of a dominant %%%epitope%%% within the %%%human%%% herpesvirus 8 ORF 65-encoded protein
AUTHOR: Pau Chou-Pong (Reprint); Lam Lee L; Spira Thomas J; Black Jodi B; Stewart John A; Pellett Philip E; Respess Richard A
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JOURNAL: Journal of Clinical Microbiology 36 (6): p1574-1577 June, 1998 1998
MEDIUM: print
ISSN: 0095-1137
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A dominant %%%epitope%%% within the %%%human%%% herpesvirus 8 (HHV8) ORF 65-encoded protein was mapped to an 8-amino-acid (aa) sequence (RKPPSGKK (aa 162 to 169)) by an amino acid %%%replacement%%% method. Using a 14-aa peptide (P4) encompassing this %%%epitope%%% as the antigen, we developed an %%%enzyme%%% immunoassay for HHV8 antibodies. The presence of P4 antibodies in a panel of 61 %%%human%%% serum specimens- was highly correlated with biopsy-confirmed Kaposi's sarcoma. The homologous Epstein-Barr virus peptide derived from BFBR3-encoded protein did not interfere with the assay, suggesting that P4 is specific for HHV8.

8/7/25
DIALOG(R)File 5:Biosis Previews(R)

0011369895 BIOSIS NO.: 199800164142
Efficient gap repair catalyzed in vitro by an intrinsic DNA polymerase activity of %%%human%%% immunodeficiency virus type 1 integrase
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JOURNAL: Journal of Virology 72 (3): p2062-2071 March, 1998 1998
MEDIUM: print
ISSN: 0022-538X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Cleavage and DNA joining reactions, carried out by %%%human%%% immunodeficiency virus type 1 (HIV-1) integrase, are necessary to effect the covalent insertion of HIV-1 DNA into the host genome. For the integration of HIV-1 DNA into the cellular genome to be completed, short gaps flanking the integrated proviral DNA must be repaired. It has been widely assumed that host cell DNA repair enzymes are involved. Here we report that HIV-1 integrase multimers possess an intrinsic DNA-dependent DNA polymerase activity. The activity was characterized by its dependence on Mg2+, resistance to N-ethylmaleimide, and inhibition by 3'-azido-2',3'dideoxythymidine-5'-triphosphate, coumermycin A,, and pyridoxal 5'-phosphate. The %%%enzyme%%% efficiently utilized poly(dA)-oligo(dT) or self-annealing oligonucleotides as a template primer but displayed relatively low activity with gapped calf thymus DNA and no activity with poly(dA) or poly(rA)-oligo(dT). A monoclonal antibody binding specifically to an %%%epitope%%% comprised of amino acids 264 to 273 near the C terminus of HIV-1 integrase severely inhibited the DNA polymerase activity. A deletion of 50 amino acids at the C terminus of integrase drastically altered the gel filtration properties of the DNA polymerase, although the level of activity was unaffected by this mutation. The DNA polymerase efficiently extended a

whether the GM1 receptor-binding B subunit of Escherichia coli heat-labile %%%toxin%%% (EtxB) could be used to deliver class I epitopes. When a class I %%%epitope%%% was conjugated to EtxB, it was delivered into the MHC-I presentation pathway in a GM1-binding-dependent fashion and resulted in the appearance of MHC-I-%%%epitope%%% complexes at the cell surface. Importantly, we show that the efficiency of EtxB-mediated %%%epitope%%% delivery could be strikingly enhanced by incorporating, adjacent to the class I %%%epitope%%%, a 10-amino-acid segment from the C terminus of the DNA polymerase (Pol) of herpes simplex virus. The %%%replacement%%% of this 10-amino-acid segment by a heterologous sequence or the introduction of specific amino acid substitutions within this segment either abolished or markedly reduced the efficiency of class I %%%epitope%%% delivery. If the %%%epitope%%% was extended at its C terminus, EtxB-mediated delivery into the class I presentation pathway was found to be completely dependent on proteasome activity. Thus, by combining the GM1-targeting function of EtxB with the 10-amino-acid Pol segment, highly efficient delivery of exogenous epitopes into the endogenous pathway of class I antigen processing and presentation can be achieved.

9/7/3
DIALOG(R)File 5:Biosis Previews(R)

0013366948 BIOSIS NO.: 200100538787
A 12-amino-acid segment, present in type s2 but not type s1 Helicobacter pylori VacA proteins, abolishes cytotoxin activity and alters membrane channel formation
AUTHOR: McClain Mark S; Cao Ping; Iwamoto Hideki; Vinion-Dubiel Arlene D; Szabo Gabor; Shao Zhifeng; Cover Timothy L (Reprint)
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JOURNAL: Journal of Bacteriology 183 (22): p6499-6508 November, 2001 2001
MEDIUM: print
ISSN: 0021-9193
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Helicobacter pylori, a gram-negative bacterium associated with gastritis, peptic ulceration, and gastric adeno-carcinoma in humans, secretes a protein %%%toxin%%%, VacA, that causes vacuolar degeneration of epithelial cells. Several different families of H. pylori vacA alleles can be distinguished based on sequence diversity in the "middle" region (i.e., m1 and m2) and in the 5' end of the gene (i.e., s1 and s2). Type s2 VacA toxins contain a 12-amino-acid amino-terminal hydrophilic segment, which is absent from type s1 toxins. To examine the functional properties of VacA toxins containing this 12-amino-acid segment, we analyzed a wild-type s1/m1 VacA and a chimeric s2/m1 VacA protein. Purified s1/m1 VacA from H. pylori strain 60190 induced vacuolation in HeLa and Vero cells, whereas the chimeric s2/m1 %%%toxin%%% (in which the s1 sequence of VacA from strain 60190 was %%%replaced%%% with the s2 sequence from strain Tx30a) lacked detectable cytotoxic activity. Type s1/m1 VacA from strain 60190 formed membrane channels in a planar lipid bilayer assay at a significantly higher rate than did s2/m1 VacA. However, membrane channels formed by type s1 VacA and type s2 VacA proteins exhibited similar anion selectivities (permeability ratio, PCl/PNa = 5). When an equimolar mixture of the chimeric s2/m1 %%%toxin%%% and the wild-type s1/m1 %%%toxin%%% was added to HeLa cells, the chimeric %%%toxin%%% completely inhibited the activity of the s1/m1 %%%toxin%%%. Thus, the s2/m1 %%%toxin%%% exhibited a dominant-negative phenotype similar to that of a previously described mutant %%%toxin%%%, VacA-(DELTA6-27). Immunoprecipitation experiments indicated that both s2/m1 VacA and VacA-(DELTA6-27) could physically interact with a c-myc %%%epitope%%%-tagged s1/m1 VacA, which suggests that the dominant-negative phenotype results from the formation of heterooligomeric VacA complexes with defective functional activity. Despite detectable differences in the channel-forming activities and cytotoxic properties of type s1 and type s2 VacA proteins, the

conservation of type s2 sequences in many H. pylori isolates suggests that type s2 VacA proteins retain an important biological activity.

9/7/4
DIALOG(R)File 5:Biosis Previews(R)

0013140814 BIOSIS NO.: 200100312653
Structure of Cry2Aa suggests an unexpected receptor binding %%%epitope%%%
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JOURNAL: Structure (London) 9 (5): p409-417 9 May, 2001 2001
MEDIUM: print
ISSN: 0969-2126
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Background: Genetically modified (GM) crops that express insecticidal protein toxins are an integral part of modern agriculture. Proteins produced by Bacillus thuringiensis (Bt) during sporulation mediate the pathogenicity of Bt toward a spectrum of insect larvae whose breadth depends upon the Bt strain. These transmembrane channel-forming toxins are stored in Bt as crystalline inclusions called Cry proteins. These proteins are the active agents used in the majority of biorational pesticides and insect-resistant transgenic crops. Though Bt toxins are promising as a crop protection alternative and are ecologically friendlier than synthetic organic pesticides, resistance to Bt toxins by insects is recognized as a potential limitation to their application. Results: We have determined the 2.2 ANG crystal structure of the Cry2Aa protoxin by multiple isomorphous %%%replacement%%%. This is the first crystal structure of a Cry %%%toxin%%% specific to Diptera (mosquitoes and flies) and the first structure of a Cry %%%toxin%%% with high activity against larvae from two insect orders, Lepidoptera (moths and butterflies) and Diptera. Cry2Aa also provides the first structure of the proregion of a Cry %%%toxin%%% that is cleaved to generate the membrane-active %%%toxin%%% in the larval gut. Conclusions: The crystal structure of Cry2Aa reported here, together with chimeric-scanning and domain-swapping mutagenesis, defines the putative receptor binding %%%epitope%%% on the %%%toxin%%% and so may allow for alteration of specificity to combat resistance or to minimize collateral effects on nontarget species. The putative receptor binding %%%epitope%%% of Cry2Aa identified in this study differs from that inferred from previous structural studies of other Cry toxins.

9/7/5
DIALOG(R)File 5:Biosis Previews(R)

0013086191 BIOSIS NO.: 200100258030
An RGD sequence in the P2Y2 receptor interacts with alphaVbeta3 integrins and is required for Go-mediated signal transduction
AUTHOR: Erb Laurie (Reprint); Liu Jun; Ockerhausen Jonathan; Kong Qiongman; Garrad Richard C; Griffin Korey; Neal Chris; Krugh Brent; Santiago-Perez Laura I; Gonzalez Fernando A; Gresham Hattie D; Turner John T; Weisman Gary A
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JOURNAL: Journal of Cell Biology 153 (3): p491-501 April 30, 2001 2001
MEDIUM: print
ISSN: 0021-9525
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The P2Y2 nucleotide receptor (P2Y2R) contains the integrin-binding domain arginine-glycine-aspartic acid (RGD) in its first

hairpin DNA primer up to 19 nucleotides on a T20 DNA template, although addition of the last nucleotide occurred infrequently or not at all. The ability of integrase to repair gaps in DNA was also investigated. We designed a series of gapped molecules containing a single-stranded region flanked by a duplex U5 viral arm on one side and by a duplex nonviral arm on the other side. Molecules varied structurally depending on the size of the gap (one, two, five, or seven nucleotides), their content of T's or C's in the single-stranded region, whether the CA dinucleotide in the viral arm had been %%%replaced%%% with a nonviral sequence, or whether they contained 5' AC dinucleotides as unpaired tails. The results indicated that the integrase DNA polymerase is specifically designed to repair gaps efficiently and completely, regardless of gap size, base composition, or structural features such as the internal CA dinucleotide or unpaired 5'-terminal AC dinucleotides. When the U5 arm of the gapped DNA substrate was removed, leaving a nongapped DNA template-primer, the integrase DNA polymerase failed to repair the last nucleotide in the DNA template effectively. A post-gap repair reaction dia depend on the CA dinucleotide. This secondary reaction was highly regulated. Only two nucleotides beyond the gap were synthesized, and these were complementary to and dependent for their synthesis on the CA dinucleotide. We were also able to identify a specific requirement for the C terminus of integrase in the post-gap repair reaction. The results are consistent with a direct role for a heretofore unsuspected DNA polymerase function of HIV-1 integrase in the repair of short gaps flanking proviral DNA integration intermediates that arise during virus infection.

8/7/26
DIALOG(R)File 5:Biosis Previews(R)

0011102581 BIOSIS NO.: 199799736641
Cysteine residues in %%%human%%% lysosomal acid lipase are involved in selective cholesteryl esterase activity
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JOURNAL: Biochemical Journal 326 (1): p265-269 1997 1997
ISSN: 0264-6021
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: %%%Human%%% lysosomal acid lipase (LAL) catalyses the deacylation of triacylglycerol and cholesteryl esters in the acidic lysosomal compartment. Treatment of LAL with the reducing agent dithiothreitol affected the triacylglycerol and cholesteryl esterase activities differentially, suggesting the involvement of cysteine residues in determining substrate specificity. To identify the residues involved, %%%human%%% LAL cDNA, under the control of the T7 promoter and tagged with a herpes simplex virus coding %%%epitope%%%, was specifically mutated in order to introduce single amino acid substitutions of each of the six cysteine residues of mature LAL. All Cys-227 mutants showed selectively decreased activity towards cholesteryl oleate, while preserving that towards trioleylglycerol. Substitutions of Cys-236, Cys-240 and Cys-244 affected catalysis towards the two substrates to a variable degree. depending on the side chain of the amino acid introduced. The %%%replacement%%% of Cys-41 or Cys-188 did not result in the preferential cleavage of either one of the two substrates. These data indicate that Cys-227, Cys-236, Cys-240 and Cys-244 play a crucial role in determining LAL substrate specificity. We propose that these cysteine residues are involved in the hydrolysis of cholesterol ester by affecting selectively the access of this substrate to the catalytic active site. In addition, the fact that the catalytic activity is never completely abolished in cysteine mutants demonstrates that LAL is not a thiol %%%enzyme%%%.

8/7/27
DIALOG(R)File 5:Biosis Previews(R)

0011079827 BIOSIS NO.: 199799713887
Identification of epitopes of monoclonal antibodies to porcine zona pellucida 3-beta glycoprotein, a homologue of the mouse/%%%human%%% sperm receptor
AUTHOR: Afzalpurkar Abhijit; Gupta S K (Reprint)
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JOURNAL: American Journal of Reproductive Immunology 38 (1): p26-32 1997 1997
ISSN: 1046-7408
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: PROBLEM: Immunization with zona pellucida (ZP) glycoproteins leads to a block in fertility with a variable degree of ovarian dysfunction. To avoid autoimmmune oophoritis, synthetic peptides corresponding to B cell %%%epitope%%%(s) and devoid of oophoritogenic T cell epitopes as immunogens have been proposed. The main objective of the present study is to define the epitopes recognized by monoclonal antibodies (mAbs) generated against porcine ZP3-beta, a homologue of the designated primary sperm receptor in mice and humans. METHODS: A multipin synthetic peptides approach has been used to map the epitopes recognized by mAbs. Dodecapeptides with an overlap of 6 amino acids corresponding to a precursor pZP3-beta-deduced amino acid sequence (excluding the signal sequence) were synthesized on polypropylene pins and were tested for their reactivity with mAbs by %%%enzyme%%%-linked immunoadsorbent assay (ELISA). The ability of synthetic peptides corresponding to the identified epitopes to inhibit the binding of mAbs to pZP3-beta in a competitive inhibition ELISA was investigated to confirm the above findings. RESULTS: Reactivity of the mAbs with the pin-bound peptides in ELISA-identified epitopes for mAb-451 to EEKLVF (166-171) and mAb-462/470 to FKAPRP (250-255) amino acid acid residues. Competitive inhibition with synthetic peptides encompassing the motifs corresponding to 23-34 and 316-321 for binding of mAb-30 to pZP3-beta revealed the epitopic domain to be 23-34 amino acids. Synthesis of overlapping octapeptides further identified WQDE as the minimum motif for binding of mAb-30, and the %%%replacement%%% of one amino acid at a time with glycine revealed tryptophan as the critical residue. CONCLUSIONS: Collectively, these results describe peptide epitopes that will help in the design of an immunocontraceptive vaccine based on synthetic peptides corresponding to pZP3-beta or its homologues in other species.

8/7/28
DIALOG(R)File 5:Biosis Previews(R)

0011070375 BIOSIS NO.: 199799704435
Transfusion requirements are correlated with the degree of proteolysis of von Willebrand factor during orthotopic liver transplantation
AUTHOR: Lattuada A; Mannucci P M (Reprint); Chen C; Legnani C; Palareti G
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JOURNAL: Thrombosis and Haemostasis 78 (2): p813-819 1997 1997
ISSN: 0340-6245
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: During orthotopic liver transplantation (OLT) excessive bleeding is the main cause of death and graft failure. The acute bleeding tendency that accompanies OLT, particularly during the anhepatic period and after reperfusion of the graft, is due to the depletion or functional abnormalities of several hemostasis components caused by the enhanced activity of enzymes such as plasmin, trypsin and leukocyte proteases. We surmised that enhanced proteolysis might also cause abnormalities of von Willebrand factor (vWF), and that these abnormalities are implicated in the bleeding tendency that develops during OLT. Therefore, the pattern of vWF proteolysis was studied with 16 patients with chronic liver disease,

in serial blood samples obtained before OLT, during the anhepatic stage, after graft reperfusion and at the end of the surgical procedure. vWF became markedly degraded during the anhepatic and reperfusion stages, as shown by the partial loss of high molecular weight multimers, the relative decrease of the intact 225 kD subunit and the increase of the native proteolytic fragments of 176 and 140 kD. Novel proteolytic fragments also became detectable. Using monoclonal antibody %%%epitope%%% mapping, it could be demonstrated that some of the proteolytic fragments corresponded in apparent molecular mass to those produced in vitro by incubating purified vWF with plasmin or elastase, but other fragments could not be attributed to these proteases. During the anhepatic and reperfusion stages there was a significant correlation between the degree of vWF degradation and the total amount of blood components transfused to %%%replace%%% blood losses. To evaluate whether or not vWF degradation could be controlled by the administration of a broad-spectrum protease inhibitor such as aprotinin, 5 patients were given a bolus dose of 500,000 U before surgery followed by 100,000 U/h during surgery, 5 were given a 2,000,000 U bolus followed by 500,060 U/h, and no aprotinin was given to the remaining 6 patients. There were no differences in the patterns or degrees of vWF degradation between patients treated with aprotinin or not. In conclusion, there is a marked degradation of a key hemostasis protein during OLT. These alterations may be of clinical significance, because they are correlated with the transfusion requirements.

8/7/29
DIALOG(R)File 5:Biosis Previews(R)

0010929987 BIOSIS NO.: 199799564047
Cleavage site for sterol-regulated protease localized to a Leu-Ser bond in the lumenal loop of sterol regulatory element-binding protein-2
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JOURNAL: Journal of Biological Chemistry 272 (19): p12778-12785 1997 1997
ISSN: 0021-9258
DOCUMENT TYPE: Article
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LANGUAGE: English

ABSTRACT: A sterol-regulated protease initiates release of the NH-2-terminal segments of sterol regulatory element-binding proteins (SREBPs) from cell membranes, thereby allowing them to enter the nucleus and to stimulate transcription of genes involved in the uptake and synthesis of cholesterol and fatty acids. Using SREBP-2 as a prototype, we here identify the site of sterol-regulated cleavage as the Leu-522-Ser-523 bond in the middle of the 31-residue hydrophilic loop that projects into the lumen of the endoplasmic reticulum and nuclear envelope. This site was identified through use of a vector encoding an SREBP-2/Ras fusion protein with a triple %%%epitope%%% tag that allowed immunoprecipitation of the cleaved COOH-terminal fragment. The NH-2 terminus of this fragment was pinpointed by radiochemical sequencing after %%%replacement%%% of selected codons with methionine codons and labeling the cells with (35S)methionine. Alanine scanning mutagenesis revealed that only two amino acids are necessary for recognition by the sterol-regulated protease: 1) the leucine at the cleavage site (leucine 522), and 2) the arginine at the P4 position (arginine 519). These define a tetrapeptide sequence, RXXL, that is necessary for cleavage. Cleavage was not affected when the second transmembrane helix of SREBP-2 was %%%replaced%%% with the membrane-spanning region of the low density lipoprotein receptor, indicating that this sequence is not required for regulation. Glycosylation-site insertion experiments confirmed that leucine 522 is located in the lumen of the endoplasmic reticulum. We conclude that the sterol-regulated protease is a novel %%%enzyme%%% whose active site faces the lumen of the nuclear envelope, endoplasmic reticulum, or another membrane organelle to which the SREBPs may be transported before cleavage.

8/7/30
DIALOG(R)File 5:Biosis Previews(R)

0010843447 BIOSIS NO.: 199799477507
The HSD-hCG vaccine prevents pregnancy in women: Feasibility study of a reversible safe contraceptive vaccine
AUTHOR: Talwar G P (Reprint); Singh Om; Gupta S K; Hasnain S E; Pal R; Majumdar S S; Vrati S; Mukhopadhay A; Srinivasan J; Deshmukh U; Ganga S; Mandokhot A; Gupta A
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JOURNAL: American Journal of Reproductive Immunology 37 (2): p153-160 1997
1997
ISSN: 1046-7408
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: PROBLEM: To develop a vaccine for reversible control of fertility in women. MATERIALS AND PROTOCOLS: Purified beta subunit of hCG annealed to purified alpha subunit of ovine LH linked chemically to tetanus toxoid (TT) and diphtheria (DT); vaccine employed at 300 mu-g gonadotropin equivalent per injection adsorbed on alhydrogel with 1 mg SPLPS added in the first injection; Phase I safety trials in 47 women with elective tubal ligation; Phase II efficacy studies in 148 proven fertile women (2 children), sexually active, desirous of family planning using IUD; IUD removed when anti-hCG titres exceed 50 ng/ml hCG bioneutralization capacity; boosters given to maintain above threshold antibody levels; post coital tests conducted in 8 volunteers; sera of protected women analysed for immuno-determinants recognized by competitive %%%enzyme%%% immunoassays employing a panel of monoclonal antibodies and by direct binding to synthetic peptides; recombinant vaccines expressing beta-hCG as a secreted product or as a fused protein anchored on membrane. RESULTS: Immunization was well tolerated with no significant changes in endocrine, metabolic and hematological indices. Normal ovulatory cycles were maintained as indicated by menstrual regulation. The vaccine was highly effective in preventing pregnancy (1 pregnancy in 1224 cycles) at and above antibody titres of 50 ng/ml. Antibodies declined in course of time in absence of boosters, with conceptions occurring below 35 ng/ml titres indicating regain of fertility. Ability of antibodies to prevent pregnancy was confirmed by post coital tests. High avidity (10-10 M-1) and other characteristics of antibodies generated by the vaccine are described and compared with those induced by two other hCG vaccines having undergone Phase I trials. The antibody response of the HSD vaccine in humans is characterized predominantly to an %%%epitope%%% recognized by the monoclonals 206 and P-3W-80. The antibodies had low or no reactivity with the carboxy terminal peptide and 38-57 region peptide. Live recombinant vaccines expressing beta-hCG as a membrane anchored peptide generated antibody response to hCG in all animals following a single injection. CONCLUSIONS: Reversible fertility control is feasible with the HSD-hCG vaccine without impairment of ovulation or disturbance of menstrual regularity. Suggestions have been made for further optimization of the vaccine, which include %%%replacement%%% of TT and DT by a panel of T non B determinants communicating with the entire MHC spectrum and development of recombinant vaccine expressing beta-hCG along with membrane anchored carrier.

8/7/31
DIALOG(R)File 5:Biosis Previews(R)

0010597475 BIOSIS NO.: 199699231535
Proline-rich tandem repeats of antibody complementarity-determining regions bind and neutralized %%%human%%% immunodeficiency virus type 1 particles
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JOURNAL: Journal of Virology 70 (10): p6557-6562 1996 1996
ISSN: 0022-538X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The proline-rich tandem repeat domain of %%%human%%% mucin MUC1 forms an extended structure containing large repeating loops that are crested by a turn. We show that the repeating-loop structure of MUC1 can be %%%replaced%%% by an antibody complementarity-determining region loop of a %%%human%%% immunodeficiency virus type 1 (HIV-1)-specific neutralizing antibody to create a chimeric, multivalent, mucin-like, anti-HIV-1 compound. We used 8 residues of an antibody molecule to %%%replace%%% 8 of 20 residues of the MUC1 tandem-repeat sequence. The antiviral peptide discussed here contains three copies of a 20-residue tandem repeat, (IYYDYEEDPAPGSTAPPAHG)-3, for a total of 60 residues. We demonstrate that the mucin-antibody chimera retains the binding specificity of the parent antibody (monoclonal antibody F58), GPGR of the HIV-1 gp120 V3 neutralizing %%%epitope%%%, and the ability to neutralize virus particles. In inhibition %%%enzyme%%%-linked immunosorbent assay, the mucin-antibody chimeric peptide could inhibit 71 to 84% of binding to a V3 loop peptide by monoclonal antibodies known to be specific for GPGR in the V3 loop. The mucin-antibody chimeric peptide could also inhibit monoclonal antibody binding to native gp120 captured from virus particles. In addition, the chimeric peptide neutralized the homologous HIV-IIIB virus in a standard neutralization assay. The methods of antiviral peptide design and construction presented here are general and theoretically limited only by the size of the antibody repertoire. This approach could be used to synthesize peptides for a variety of therapeutic applications.

8/7/32
DIALOG(R)File 5:Biosis Previews(R)

0010447891 BIOSIS NO.: 199699081951
Crystal structure of %%%human%%% trypsin 1: Unexpected phosphorylation of Tyr151
AUTHOR: Gaboriaud Christine (Reprint); Serre Laurence; Guy-Crotte Odette; Forest Eric; Fontecilla-Camps Juan-Carlos
AUTHOR ADDRESS: Lab. de Cristallographie et Cristallogenese des Proteines, Inst. de Biologie Structurale Jean-Pierre Ebel, 41 Avenue des Martyrs, 30827 Grenoble Cedex 1, France**France
JOURNAL: Journal of Molecular Biology 259 (5): p995-1010 1996 1996
ISSN: 0022-2836
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The X-ray structure of %%%human%%% trypsin 1 has been determined in the presence of diisopropyl-phosphofluoridate by the molecular %%%replacement%%% method and refined at a resolution of 2.2 ANG to an R-factor of 18%. Crystals belong to the space group P4, with two independent molecules in the asymmetric unit packing as crystallographic tetramers. This study was performed in order to seek possible structural peculiarities of %%%human%%% trypsin 1, suggested by some striking differences in its biochemical behavior as compared to other trypsins of mammalian species. Its fold is, in fact, very similar to those of the bovine, rat and porcine trypsins, with root-mean-square differences in the 0.4 to 0.6 ANG range for all 223 C-alpha positions. The most unexpected feature of the %%%human%%% trypsin 1 structure is in the phosphorylated state of tyrosine residue 151 in the present X-ray study. This feature was confirmed by mass spectrometry on the same inhibited sample and also on the native %%%enzyme%%%. This phosphorylation strengthens the outstanding clustering of highly negative or highly positive electrostatic surface potentials. The peculiar inhibitory behaviour of pancreatic secretory trypsin inhibitors of the Kazal type on this %%%enzyme%%% is discussed as a possible consequence of these properties. A charged surface loop has also been interpreted as an %%%epitope%%% site recognised by a monoclonal antibody specific to

%%%human%%% trypsin 1.

8/7/33
DIALOG(R)File 5:Biosis Previews(R)

0010031378 BIOSIS NO.: 199598499211
Presentation of HIV V3 lõop epitopes for enhanced antigenicity, immunogenicity and diagnostic potential
AUTHOR: Fontenot J Darrell (Reprint); Vancott Tom C; Parekh Bharat S; Pau Chou-Pong; George J Richard; Birx Debra L; Zolla-Pazner Susan; Gorny Miroslaw K; Gatewood Jose M
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JOURNAL: AIDS (Philadelphia) 9 (10): p1121-1129 1995 1995
ISSN: 0269-9370
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Objective: To evaluate the immunological properties of a panel of %%%human%%% mucin MUC1/HIV V3 loop chimeras. Design: The immunodominant %%%epitope%%% of MUC1 (APDTR) was found to be structurally isomorphous with the tip of the principle neutralizing determinant (PND) of HIV-1 (MN) (GPGRA). A panel of 120 residue, six tandem repeat (TR) and 60 residue, three TR chimeric antigens were constructed in which the repeating MUC1 %%%epitope%%% is %%%replaced%%% by HIV-1 PND. Each 20 residue TR contains one PND %%%epitope%%%. The PND of HIV-1 is presented in the native beta-turn conformation at the crest of each repeating knob structure of the mucin-like protein. Methods: The antigenicity of the chimeric antigens were compared using %%%enzyme%%%-linked immunosorbent assay (ELISA) and HIV-infected patient sera. Structural effects of antibody-antigen interactions were determined using surface plasmon resonance, with %%%human%%% monoclonal antibodies, chimeric antigens and the cyclic and linear V3 loops. Immunogenicity of three versus six TR was measured in mice. Results: Nine residues of the HIV PND substituted into the mucin backbone were equivalent to the 36 residue cyclic V3 loop in ELISA. The 120 residue antigens induced high titer, immunoglobulin (Ig) M and IgG, and HIV-specific antibodies in mice. Conclusions: MUC1/V3 chimeras efficiently detect HIV-specific antibodies in patient sera. Multivalent presentation of the PND is advantageous for higher affinity antibody-antigen interactions and for inducing HIV-specific IgM and IgG antibodies.

8/7/34
DIALOG(R)File 5:Biosis Previews(R)

0009892232 BIOSIS NO.: 199598360065
An approach for an immunoaffinity AIDS sensor using the conservative region of the HIV envelope protein (gp41) and its monoclonal antibody
AUTHOR: Uda Taizo (Reprint); Hifumi Emi; Kobayashi Toshiaki; Shimizu Kosuke ; Sata Tetsutaro; Ogino Keiki
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JOURNAL: Biosensors and Bioelectronics 10 (5): p477-483 1995 1995
ISSN: 0956-5663
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A monoclonal antibody for the conservative region of gp41, which is one of the HIV envelope proteins, was produced. The antigen determining site of gp41 was examined using the %%%epitope%%% mapping technique, followed by an %%%enzyme%%% linked immunosorbent assay. Some peptides had comparable affinities for the monoclonal antibody, but the peptide EGIEE, having a slightly weaker immunoaffinity than gp41, was the most preferable for the construction of an immunoaffinity AIDS sensor. For the detection of gp41, EGIEE was labelled with catalase and used as a

mimic antigen; it was bound to the antibody present on an immuno-membrane and, due to the %%%replacement%%% reaction of the mimic antigen by gp41, indirect quantitative measurement of gp41 was possible using an oxygen electrode. Anti-gp41 antibody was also detected using a mimic antibody, which was chemically modified with polyethylene glycol. An immunoaffinity AIDS sensor was constructed using the mimic molecules which were tailored to have a suitable immunoaffinity for HIV antigen and/or antibody.

8/7/35
DIALOG(R)File 5:Biosis Previews(R)

0009765830 BIOSIS NO.: 199598233663
Acceptor specificity of different length constructs of %%%human%%% recombinant alpha-1,3/4-fucosyltransferase: %%%Replacement%%% of the stem region and the transmembrane domain of fucosyltransferase V by protein A results in an %%%enzyme%%% with GDP-fucose hydrolyzing activity
AUTHOR: De Vries Theodora (Reprint); Srnka Cheryl A; Palcic Monica M; Swiedler Stuart J; Van Den Eijnden Dirk H; Macher Bruce A
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JOURNAL: Journal of Biological Chemistry 270 (15): p8712-8722 1995 1995
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The acceptor specificity of recombinant full-length, membrane-bound fucosyltransferases, expressed in COS-7 cells, and soluble, protein-A chimeric forms of alpha-1,3-fucosyltransferase (Fuc-T) III, Fuc-TIV, and Fuc-TV was analyzed toward a broad panel of oligosaccharide, glycolipid, and glycoprotein substrates. Our results on the full-length enzymes confirm and extend previous studies. However, chimeric Fuc-Ts showed increased activity toward glycoproteins, whereas chimeric Fuc-TIII and Fuc-TV had a decreased activity with glycosphingolipids, compared to the full-length enzymes. Unexpectedly, chimeric Fuc-TV exhibited a GDP-fucose hydrolyzing activity. In substrates with multiple acceptor sites, the preferred site of fucosylation was identified. Fuc-TIII and Fuc-TV catalyzed fucose transfer exclusively to OH-3 of glucose in lacto-N-neotetraose and lacto-N-tetraose, respectively, as was demonstrated by 1H NMR spectroscopy. Thin layer chromatography immunostaining revealed that FucT-IV preferred the distal GlcNAc residue in nLc-6Cer, whereas Fuc-TV preferred the proximal GlcNAc residue. Incubation of Fuc-TIV or Fuc-TV with VI-3NeuAcnLc-6Cer resulted in products with the sialyl-Lewis-x %%%epitope%%% as well as the VIM-2 structure. To identify polar groups on acceptors that function in %%%enzyme%%% binding, deoxygenated substrate analogs were tested as acceptors. All three Fuc-Ts had an absolute requirement for a hydroxyl at C-6 of galactose in addition to the accepting hydroxyl at C-3 or C-4 of GlcNAc.

8/7/36
DIALOG(R)File 5:Biosis Previews(R)

0008876070 BIOSIS NO.: 199396040486
Comparison of a whole-virus %%%enzyme%%% immunoassay (EIA) with a peptide-based EIA for detecting rubella virus immunoglobulin G antibodies following rubella vaccination
AUTHOR: Zrein Maan (Reprint); Joncas Jean H; Pedneault Louise; Robillard Louise; Dwyer Robert J; Lacroix Martial
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JOURNAL: Journal of Clinical Microbiology 31 (6): p1521-1524 1993
ISSN: 0095-1137
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A total of 250 %%%human%%% serum samples were tested for rubella

virus immunoglobulin G antibodies by two %%%enzyme%%% immunoassays (EIAs), one using whole rubella virus antigen and the other based on the use of synthetic peptide antigen. The samples were taken from 125 volunteers before and after their immunization with the RA 27/3 rubella vaccine. This study indicates that a synthetic peptide-based EIA can favorably %%%replace%%% current viral lysate-based EIAs to detect rubella virus antibodies following immunization. Because the synthetic peptide used in this newly developed EIA represents a putative neutralization %%%epitope%%% of the rubella virus, it could also be instrumental in determining rubella immune status and in assessing vaccine program efficiency.

8/7/37
DIALOG(R)File 5:Biosis Previews(R)

0008865397 BIOSIS NO.: 199396029813
Identification of heat-stable enterotoxin-producing strains of Yersinia enterocolitica and Vibrio cholerae non-O1 by a monoclonal antibody-based %%%enzyme%%%-linked immunosorbent assay
AUTHOR: Nair G Balakrish; Bhattacharya Sujit K; Takeda Tae (Reprint)
AUTHOR ADDRESS: Dep. Infectious Diseases Res., Natl. Children's Med. Res. Center, 3-35-31 Taishido, Setagaya-ku, Tokyo 154, Japan**Japan
JOURNAL: Microbiology and Immunology 37 (3): p181-186 1993
ISSN: 0385-5600
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Using a mouse monoclonal antibody (MAb) 2F raised against Vibrio cholerae non-O1 heat-stable enterotoxin (NAG-ST) which also recognizes a shared %%%epitope%%% of Yersinia enterocolitica heat-stable enterotoxin (Y-ST), a competitive %%%enzyme%%%-linked immunosorbent assay (ELISA) was developed for independent detection of NAG-ST and Y-ST. There was good concordance between the Y-ST ELISA and the suckling mouse assay (SMA) for detection of Y-ST from test strains of Y. enterocolitica, and the Y-ST ELISA can effectively %%%replace%%% the SMA for routine detection of Y-ST. On the contrary, evaluation of the NAG-ST ELISA and the SMA using 139 strains of V. cholerae non-O1 showed discordant results and this was attributed to the presence of the suckling mice active factor(s) such as El Tor hemolysin and to the production of low amounts of NAG-ST. Concentration of culture supernatants of V. cholerae non-O1 followed by heating at 100 C was essential to obtain reproducible results by both the NAG-ST ELISA and the SMA. The ELISA developed in this study can be used for the identification of biologically active strains. While recently genetic methods such as polymerase chain reaction became available and were very reliable and simple techniques, the ELISA in this study has an advantage in detecting biologically toxic gene products of the strains. The genetic methods cannot differentiate silent STa genes which we often encounter in the case of Y. enterocolitica.

8/7/38
DIALOG(R)File 5:Biosis Previews(R)

0008326918 BIOSIS NO.: 199294028759
IGG ANTIBODY RESPONSE TO POLYETHYLENE GLYCOL-MODIFIED ADENOSINE DEAMINASE IN PATIENTS WITH ADENOSINE DEAMINASE DEFICIENCY
AUTHOR: CHAFFEE S (Reprint); MARY A; STIEHM E R; GIRAULT D; FISCHER A; HERSHFIELD M S
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JOURNAL: Journal of Clinical Investigation 89 (5): p1643-1651 1992
ISSN: 0021-9738
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Polyethylene glycol (PEG)-modified bovine adenosine deaminase (ADA) is used for %%%replacement%%% therapy of severe combined

immunodeficiency disease due to inherited ADA deficiency. We monitored IgG anti-ADA antibody in 17 patients treated by intramuscular injections of PEG-ADA for 1 to > 5.5 yr. ELISA-detectable anti-ADA IgG appeared in 10 patients, usually between the third and eighth months of treatment. Anti-ADA level did not correlate with trough plasma ADA activity, which averaged 1.8-5 times normal blood (erythrocyte) ADA activity, depending on dose (15-60 U/kg per wk). ELISA-detectable anti-ADA antibodies were directed primarily at bovine-specific (rather than PEG-containing) epitopes. Enhanced %%%enzyme%%% clearance, mediated by antibody that directly inhibited native and PEG-modified bovine ADA, and native, but not PEG-modified %%%human%%% ADA, occurred in two patients. In one, tolerance was induced; in the second, twice weekly injections of PEG-ADA compensated for accelerated clearance. We speculate that inhibitory antibodies recognize conserved, relatively PEG-free %%%epitope%%%(s) encompassing the active site, and that in %%%human%%%, but not bovine, ADA a PEG-attachment site "shields" the active site from immune recognition. We conclude that Peg-modification largely prevents the development of high affinity, or high levels of, clearing antibodies to bovine ADA, and that PEG-modified %%%human%%% ADA should be further investigated as a possible treatment for ADA deficiency.

8/7/39
DIALOG(R)File 5:Biosis Previews(R)

0007748709 BIOSIS NO.: 199191131600
ANALYSIS OF N ACETYLGALACTOSAMINE-4-SULFATASE PROTEIN AND KINETICS IN MUCOPOLYSACCHARIDOSIS TYPE VI PATIENTS
AUTHOR: BROOKS D A (Reprint); MCCOURT P A G; GIBSON G J; ASHTON L J; SHUTTER M; HOPWOOD J J
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JOURNAL: American Journal of Human Genetics 48 (4): p710-719 1991
ISSN: 0002-9297
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: A sensitive and specific, monoclonal antibody-based immunoquantification assay has facilitated determination of the N-acetylgalactosamine-4-sulfatase (4-sulfatase) protein content in cultured fibroblasts from normal controls and mucopolysaccharidosis type VI (MPS VI) patients. The assay enabled the quantification of 4-sulfatase protein by using a panel of seven monoclonal antibodies and has shown that fibroblasts from 16 MPS VI patients contained .ltoreq. 5% of the level determined for normal controls. Fibroblasts from the most severely affected patients contained the lowest levels of 4-sulfatase protein, usually with few epitopes detected, while fibroblasts from mildly affected patients had higher levels of 4-sulfatase protein, with all seven epitopes detected. The pattern of %%%epitope%%% expression is proposed to reflect the conformational changes in the 4-sulfatase protein that arise from different mutations in the 4-sulfatase gene. Immunoquantification in combination with a specific and highly sensitive 4-sulfated trisaccharide-based assay of %%%enzyme%%% activity in these MPS VI patient fibroblasts enabled the determination of residual 4-sulfatase catalytic efficiency (kcat/Km). The capacity of fibroblasts to degrade substrate (catalytic capacity) was calculated as the product of 4-sulfatase catalytic efficiency and the content of 4-sulfatase in fibroblasts. One patient, 2357, with no clinical signs of MPS VI but with reduced 4-sulfatase activity and protein (both 5% of normal) and dermatansulfaturia, had 5% of normal catalytic capacity. The other 15 MPS VI patient fibroblasts had 0%-1.4% of the catalytic capacity of fibroblasts from normal controls and were representative of the spectrum of MPS VI clinical phenotypes, from severe to mild. It is proposed that an %%%enzyme%%%-%%%replacement%%% therapy achieving a correction of > 5% of normal catalytic capacity is required to avoid the onset of MPS VI clinical phenotype. Moreover, it is sugested that the restored catalytic capacity must be correctly localized within the lysosomal compartment to enable effective treatment of MPS VI.

8/7/40
DIALOG(R)File 5:Biosis Previews(R)

0007687666 BIOSIS NO.: 199191070557
%%%HUMAN%%% SALIVARY PEROXIDASE AND BOVINE LACTOPEROXIDASE ARE CROSS-REACTIVE
AUTHOR: MANSSON-RAHEMTULLA B (Reprint); RAHEMTULLA F; HUMPHREYS-BEHER M G
AUTHOR ADDRESS: DEP COMMUNITY PUBLIC HEALTH DENTISTRY, UNIVERSITY ALABAMA SCHOOL DENTISTRY, UAB STATION, BIRMINGHAM, ALA 35294, USA**USA
JOURNAL: Journal of Dental Research 69 (12): p1839-1846 1990
ISSN: 0022-0345
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Peroxidases are abundant in nature, and the primary function of mammalian peroxidases is to catalyze the peroxidation of halides and pseudohalides. Previous studies have shown that antibodies raised against bovine lactoperoxidase moderately cross-react with %%%human%%% salivary peroxidase, a feature that has been used in the present study to examine epitopes common to the antigen and %%%human%%% salivary peroxidase. Polyclonal antibodies against a highly purified preparation of bovine lactoperoxidase were raised in rabbits, and their properties were examined. In double-immunodiffusion experiments, the two enzymes showed partial identity, and in competitive radioimmunoassay and %%%enzyme%%% -linked immunosorbent assay, lactoperoxidase %%%replaced%%% the labeled and coated antigen, while salviary peroxidase did not. However, salivary peroxidase from %%%human%%% and rat saliva samples and the purified %%%enzyme%%% in its non-reduced, reduced, and de-glycosylated forms were recognized by these antibodies, as analyzed by Western blot analysis and immunodetection. The major activity of these antibodies was directed against the protein core of the antigen. Immunodetection of the peptide fragments of bovine lactoperoxidase and %%%human%%% salivary peroxidase revealed structural differences in the two enzymes. These antibodies also precipitated an in vitro translation product from rat-parotid-gland cell lysate that, on SDS-PAGE, compared favorably with the expected molecular weight of a de-glycosylated peroxidase. The antibodies partly inhibited the %%%enzyme%%% activity of salivary peroxidase and the peroxidase in rat parotid gland lysate, but the %%%enzyme%%% activity of lactoperoxidase was not affected by addition of antilactoperoxidase IgG between 25 and 400 .mu.g/mL. The %%%enzyme%%% activity remained unchanged in all samples when pre-immune IgG was used.

8/7/41
DIALOG(R)File 5:Biosis Previews(R)

0007679068 BIOSIS NO.: 199191061959
SITE-DIRECTED MUTAGENESIS OF LYSINE WITHIN THE IMMUNODOMINANT AUTOEPITOPE OF PDC-E2
AUTHOR: LEUNG P S C (Reprint); IWAYAMA T; COPPEL R L; GERSHWIN M E
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JOURNAL: Hepatology 12 (6): p1321-1328 1990
ISSN: 0270-9139
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: The major autoantigens of PBC have been identified as the four closely related mitochondrial enzymes PDC-E2, BCKD-E2 OGDC-E2 and protein X. A major structural similarly to these enzymes is the presence of one or more lipoyl domains. The immunodominant %%%epitope%%% of each autoantigen has either been postulated or been demonstrated to be located within the lipoate binding region. However, it is not clear whether the binding of lipoic acid to the %%%epitope%%% is necessary for autoantibody recognition. To address this issue we have constructed by oligonucleotide site-directed mutagenesis three mutants in the lipoyl domain of

%%%human%%% PDC-E2. Because lipoic acid is covalently bound to the .epsilon.-amino group of the lysine residue of PDC-E2, the mutants were designed to %%%replace%%% the lysine residue in the lipoyl domain with glutamine, a negatively charged amino acid; histidine, a positively charged amino acid; and tyrosine, an aromatic amino acid. Binding reactivity of sera from patients with PBC were analyzed by %%%enzyme%%% -linked immunosorbent assay, immunoblotting and specific absorption against each of the three mutants and control clones. All data were compared with parallel studies with a control recombinant clone, the liver-specific F alloantigens. We believe the recognition of the lipoyl domain is a reflection of the surface-exposed, hydrophilic and relatively mobile nature of this region of the autoantigen. Further studies on direct assay for the presence of lipoic acid will be needed to clarify these issues.

8/7/42
DIALOG(R)File 5:Biosis Previews(R)

0006805830 BIOSIS NO.: 198988120945
DEVELOPMENT OF AN %%%ENZYME%%% IMMUNOASSAY USING RECOMBINANT EXPRESSED ANTIGEN TO DETECT HEPATITIS DELTA VIRUS ANTIBODIES
AUTHOR: PUIG J (Reprint); FIELDS H A
AUTHOR ADDRESS: HEPATIS BRANCH, WORLD HEALTH ORGANIZATION COLLABORATING CENT REFERENCE, DIV VIRAL DIS, CENT DIS CONTROL, ATLANTA, GA 30333, USA** USA
JOURNAL: Journal of Clinical Microbiology 27 (10): p2222-2225 1989
ISSN: 0095-1137
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Two generic %%%enzyme%%% immuonassays (EIAs) were developed for detection of anti-hepatitis delta virus antibodies (anti-HD) and compared with a commercially available radioimmunoassay. Both generic assays were configured as blocking assays and used hepatitis delta antigen (HDAg) derived from infected chimpanzee liver (EIA-1) or from Escherichia coli transformed with a plasmid containing an insert from within an open reading frame encoding HDAg (EIA-2). Absolute sensitivity was ascertained by endpoint titration, which demonstrated essentially identical endpoints for EIA-1 and EIA-2. The absolute sensitivities of the EIAs were approximately four times greater than that of the radioimmunoassay. Specificity and sensitivity were ascertained by testing a panel of 176 serum specimens by each assay. The specimens were selected to represent a panel composed of sera from individuals with or without markers of viral hepatitis as follows: (i) serologically confirmed by exclusion as posttransfusion non-A, non-B hepatitis; (ii) acute or chronic hepatitis B virus infection, positive for hepatitis B surface antigen; (iii) resolved hepatitis B virus infection, positive for anti-hepatitis B surface antigen; (iv) acute hepatitis A virus infection, positive for anti-hepatitis A virus immunoglobulin M; and (v) normal %%%human%%% sera. All three assays for anti-HD gave similar specificity and sensitivity values. In conclusion, the recombinant expressed HDAg can %%%replace%%% antigen derived from infected liver tissue as a diagnostic reagent used to configure an EIA for detection of anti-HD. Furthermore, the results suggest that the expressed antigen contains the important immunodominant %%%epitope%%%(s).

8/7/43
DIALOG(R)File 5:Biosis Previews(R)

0006734886 BIOSIS NO.: 198988050001
ACQUIRED C1 INHIBITOR C1-INH DEFICIENCY TYPE II %%%REPLACEMENT%%% THERAPY WITH C1-INH AND ANALYSIS OF PATIENTS' C1-INH AND ANTI-C1-INH AUTOANTIBODIES
AUTHOR: ALSENZ J (Reprint); LAMBRIS J D; BORK K; LOOS M
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JOURNAL: Journal of Clinical Investigation 83 (6): p1784-1799 1989
ISSN: 0021-9738
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: The response of two patients with autoantibody-mediated C1-inhibitor (C1-INH) deficiency to %%%replacement%%% therapy with C1-INH was studied over a period of 3 d. In patient 1 an acute attack of angioedema was successfully managed by infusion of 1,000 U of C1-INH concentrate. C1-INH function returned to normal levels within 30 min, while CH50 and C4 peaked after 6-7 h and C1 hemolytic activity reached 50-60% of normal after 3 d. Immediately after the injection an increase in C1-INH-anti-C1-INH complexes was observed. Based on NH2-terminal sequence analysis of the patients' Mr 96,000 C1-INH, it is concluded that this fragment is generated after cleavage of C1-INH in its active site by one of its target proteases without generating a covalent C1-INH-%%%enzyme%%% complex. In a second patient with a four to five times higher anti-C1-INH antibody titer, the infusion of 500 ml of plasma or of 2,000 U of C1-INH concentrate influenced neither the severity of the patient's angioedema nor the tested parameters, except for an increase in the amount of C1-INH-anti-C1-INH complexes. Analysis of patients' anti-C1-INH antibodies revealed that the antibodies recognize different epitopes within the C1-INH. This suggests that patients with acquired angioedema type II are a heterogenous group with respect to the C1-INH autoantibodies.

8/7/44
DIALOG(R)File 5:Biosis Previews(R)

0005745013 BIOSIS NO.: 198784099162
ELISA FOR DETECTION OF ANTIBODIES TO THE VENEREAL DISEASE RESEARCH LABORATORY VDRL ANTIGEN IN SYPHILIS
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AUTHOR ADDRESS: WORLD HEALTH ORGANIZATION COLLABORAING CENTRE REFERENCE RES TREPONEMATOSE, DEP TREPONEMATOSE, DK-2300 COPENHAGEN S DENMARK**DENMARK
JOURNAL: Journal of Clinical Microbiology 25 (9): p1711-1716 1987
ISSN: 0095-1137
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: An %%%enzyme%%%-linked immunosorbent assay (ELISA) for detection of immunoglobulin G (IgG) and IgM to cardiolipin, lecithin, and cholesterol (VDRL [Veneral Disease Research Laboratory] ELISA) is described. The specificity of the VDRL ELISA for IgG and IgM was 99.6 and 99.5%, respectively, with sera from 1,008 persons without syphilis. For a group of patients with false-positive results in traditional nontreponemal tests and for patients with autoimmune diseases, the VDRL ELISA for IgG had a higher specificity than the VDRL ELISA for IgM. The sensitivity for IgG and IgM with 118 sera from patients with untreated syphilis was 96.6 and 94.9%, respectively, which was equivalent to the sensitivities of the traditional nontreponemal tests. The performance of the VDRL ELISA was compared with that of an ELISA that uses cardiolipin as the antigen (cardiolipin ELISA). The VDRL ELISA was significantly more sensitive (P .ltoreq. 0.01) than the cardiolipin ELISA with 25 sera from syphilis patients but was less sensitive (P .ltoreq. 0.01) with 53 sera from patients with autoimmune diseases. The antibody reactivity in the VDRL ELISA could not be absorbed out by lecthin and cholesterol, and the sera from patients with syphilis did not react in an ELISA that uses cholesterol and lecithin as the antigen. This indicates that cholesterol and lecithin, although not antigenic by themselves, may change the structural form of the %%%epitope%%% on cardiolipin so that it becomes more recognizable for antibodies in syphilis and less recognizable for antibodies in autoimmune diseases. The results of the VDRL ELISA were expressed in percentages of the absorbance value of a positive control. The VDRL ELISA gave, without titration of sera, quantitative results that correlated with the quantitative results of the traditional nontreponemal tests obtained by titration. The VDRL ELISA will be well suited for

large-scale testing for syphilis and may %%%replace%%% other nontreponemal tests.

toxin epitope replacement

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9/7/1
DIALOG(R)File 5:Biosis Previews(R)

0014138816 BIOSIS NO.: 200300097535
Effect of amino acid substitutions in the %%%epitope%%% regions of pyolysin from Arcanobacterium pyogenes.
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JOURNAL: Veterinary Microbiology 91 (2-3): p205-213 2 February, 2003 2003
MEDIUM: print
ISSN: 0378-1135
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Pyolysin (PLO), secreted by Arcanobacterium pyogenes, is a novel member of the thiol-activated cytolysin (TACY, cholesterol-dependent cytolysin) family of bacterial toxins. Recently, we demonstrated that the epitopes of monoclonal antibodies (mAbs) S, H, C, and G lie in the regions of amino acids regions 55-73, 123-166, 482-506, and 482-506 of PLO, respectively, by the reaction of mAbs with truncated PLOs. In this study, we substituted the amino acids in these %%%epitope%%% regions of PLO by site-directed mutagenesis and examined the effect of these amino acid substitutions. Mutants 170S/R71A/L73S, Y131S/P132S, and L163S/P164S for mAbs H or S completely lost the hemolytic activity of the proteins, but these mutants still bound to erythrocyte membranes. Mutants L495S/W497S and W500S/W501S for mAbs C and G also completely lost their hemolytic activity, but still bound to erythrocyte membranes. In the undecapeptide region of PLO, the cysteine residue required for thiol activation is %%%replaced%%% with alanine. Therefore, we substituted Ala-492 of the undecapeptide region for Cys. The hemolytic activity of this mutant A492C decreased by adding hydrogen peroxide or storing at 4degreeC, and the decreased hemolytic activity was restored by adding L-cysteine.

9/7/2
DIALOG(R)File 5:Biosis Previews(R)

0013763475 BIOSIS NO.: 200200356986
Enhanced delivery of exogenous peptides into the class I antigen processing and presentation pathway
AUTHOR: de Haan Lolke; Hearn Arron R; Rivett A Jennifer; Hirst Timothy R (Reprint)
AUTHOR ADDRESS: Departments of Pathology and Microbiology, School of Medical Sciences, University of Bristol, University Walk, Bristol, BS8 1TD, UK**UK
JOURNAL: Infection and Immunity 70 (6): p3249-3258 June, 2002 2002
MEDIUM: print
ISSN: 0019-9567
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Current immunization strategies, using peptide or protein antigens, generally fail to elicit cytotoxic-T-lymphocyte responses, since these antigens are unable to access intracellular compartments where loading of major histocompatibility complex class I (MHC-I) molecules occurs. In an attempt to circumvent this, we investigated

extracellular loop, raising the possibility that this G protein-coupled receptor interacts directly with an integrin. Binding of a peptide corresponding to the first extracellular loop of the P2Y2R to K562 erythroleukemia cells was inhibited by antibodies against alphaVbeta3/beta5 integrins and the integrin-associated thrombospondin receptor, CD47. Immunofluorescence of cells transfected with %%%epitope%%%-tagged P2Y2Rs indicated that alphaV integrins colocalized 10-fold better with the wild-type P2Y2R than with a mutant P2Y2R in which the RGD sequence was %%%replaced%%% with RGE. Compared with the wild-type P2Y2R, the RGE mutant required 1,000-fold higher agonist concentrations to phosphorylate focal adhesion kinase, activate extracellular signal-regulated kinases, and initiate the PLC-dependent mobilization of intracellular Ca2+. Furthermore, an anti-alphaV integrin antibody partially inhibited these signaling events mediated by the wild-type P2Y2R. Pertussis %%%toxin%%%, an inhibitor of Gi/o proteins, partially inhibited Ca2+ mobilization mediated by the wild-type P2Y2R, but not by the RGE mutant, suggesting that the RGD sequence is required for P2Y2R-mediated activation of Go, but not Gq. Since CD47 has been shown to associate directly with Gi/o family proteins, these results suggest that interactions between P2Y2Rs, integrins, and CD47 may be important for coupling the P2Y2R to Go.

9/7/6
DIALOG(R)File 5:Biosis Previews(R)

0012606992 BIOSIS NO.: 200000325305
An example of immunodominance: Engagement of synonymous TCR by invariant CDR3beta
AUTHOR: Wienhold Wolfgang (Reprint); Malcherek Georg; Jung Christine; Stevanovic Stefan; Jung Guenther; Schild Hansjoerg; Melms Arthur
AUTHOR ADDRESS: Neuroimmunology Laboratory, Department of Neurology, University of Tuebingen, 72076, Tuebingen, Germany**Germany
JOURNAL: International Immunology 12 (6): p747-756 June, 2000 2000
MEDIUM: print
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ABSTRACT: The structural basis of the T cell response against immunodominant tetanus %%%toxin%%% (TT)-derived peptides was investigated using TT-specific T cell clones raised from a DRB1*0301 homozygous donor. Three peptides forming T cell epitopes were identified, including one, TT(1272-1284), that stimulated four different TT-specific T cell clones. TCR sequence analysis revealed that these synonymous TCR shared only arginine at the third position of the CDR3beta loop. This prominent residue may form a salt bridge with a corresponding aspartate at the relative position 8 (P8) of the antigenic peptide TT(1272-1284) as suggested from amino acid %%%replacement%%% analysis. A similar scenario was observed for a second TT %%%epitope%%%, TT(279-296), and its corresponding TCR. These examples show that immunodominance may result from a single strong amino acid interaction between TCR CDR3beta loops here in contact with the C-terminus of the antigenic peptide. Such a dominant interaction could compensate for weaker contacts between other residues of the TCR and the antigenic peptide, and would allow the recognition of a single peptide-MHC complex by a broader synonymous TCR repertoire and could thus contribute to its immunodominance.

9/7/7
DIALOG(R)File 5:Biosis Previews(R)

0012095810 BIOSIS NO.: 199900355470
Design of highly immunogenic liposomal constructs combining structurally independent B cell and T helper cell peptide epitopes
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JOURNAL: European Journal of Immunology 29 (7): p2297-2308 July, 1999 1999
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: We have designed liposomal diepitope constructs that allow the physical combination, within the same vesicle, of B and Th epitopes as structurally separate entities. The immune response against such constructs was explored using TPEDPTDPTDPQDPSS (TPE), a B cell %%%epitope%%% originating from a Streptococcus mutans surface adhesin and QYIKANSKFIGITEL (QYI), a "universal" Th %%%epitope%%% from tetanus %%%toxin%%%. The two peptides were linked to the outer surface of small (diameter approximately 100 nm) unilamellar liposomes by covalent conjugation to two different anchors. To that end we have developed a strategy that allows the controlled chemical coupling of TPE and QYI, functionalized at their N terminus with a thiol, to preformed liposomes containing thiol-reactive derivatives of phosphatidylethanolamine and the lipopeptide S-(2,3-bis (palmitoyloxy)-(2-RS)-propyl)-N-palmitoyl-(R)-cysteinyl-alanyl-glycine (Pam3CAG), respectively. This synthetic construct (administered i.p. to BALB/c mice) inducedhighly intense (titers > 20 000), anamnestic and long-lasting (over 2 years) immune responses, indicating that this strategy is successful. Two parameters were of prime importance to elicit this response with our liposomal diepitope constructs: (1) the simultaneous expression of B and Th epitopes on the same vesicle, and (2) the lipopeptide Pam3CAG anchor of the Th %%%epitope%%% QYI could not be %%%replaced%%% by a phosphatidylethanolamine anchor (a lesser immune response was observed). Analysis of the antibody response revealed a complex pattern; thus, besides the humoral response (production of IgG1, IgG2a, IgG2b) a superposition of a T-independent (TI-2 type) response was also found (IgM and IgG3). These results indicate that liposomal diepitope constructs could be attractive in the development of synthetic peptide-based vaccines.

9/7/8
DIALOG(R)File 5:Biosis Previews(R)

0012081113 BIOSIS NO.: 199900340773
Soft docking an L and a D peptide to an anticholera %%%toxin%%% antibody using internal coordinate mechanics
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JOURNAL: Structure (London) 7 (6): p663-670 June, 1999 1999
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LANGUAGE: English

ABSTRACT: Background: The tremendous increase in sequential and structural information is a challenge for computer-assisted modelling to predict the binding modes of interacting biomolecules. One important area is the structural understanding of protein-peptide interactions, information that is increasingly important for the design of biologically active compounds. Results: We predicted the three-dimensional structure of a complex between the monoclonal antibody TE33 and its cholera-%%%toxin%%% -derived peptide %%%epitope%%% VPGSQHID. Using the internal coordinate mechanics (ICM) method of flexible docking, the bound conformation of the initially extended peptide %%%epitope%%% to the antibody crystal or modelled structure reproduced the known binding conformation to a root mean square deviation of between 1.9 ANG and 3.1 ANG. The predicted complexes are in good agreement with binding data obtained from substitutional analyses in which each %%%epitope%%% residue is

%%%replaced%%% by all other amino acids. Furthermore, a denovo prediction of the recently discovered TE33-binding D peptide dwGsqhydp (single-letter amino acid code where D amino acids are represented by lower-case letters) explains results obtained from binding studies with 172 peptide analogues. Conclusions: Despite the difficulties arising from the huge conformational space of a peptide, this approach allowed the prediction of the correct binding orientation and the majority of essential binding features of a peptide-antibody complex.

9/7/9
DIALOG(R)File 5:Biosis Previews(R)

0011427687 BIOSIS NO.: 199800221934
Identification of a novel heptoglycan of alpha1fwdarw2-linked D-glycero-D-manno-heptopyranose. Chemical and antigenic structure of lipopolysaccharides from Klebsiella pneumoniae ssp. Pneumoniae rough strain R20 (O1-:k20-)
AUTHOR: Suesskind Miriam; Brade Lore; Brade Helmut; Holst Otto (Reprint)
AUTHOR ADDRESS: Res. Cent. Borstel, Parkallee 22, D-23845 Borstel, Germany **Germany
JOURNAL: Journal of Biological Chemistry 273 (12): p7006-7017 March 20, 1998 1998
MEDIUM: print
ISSN: 0021-9258
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RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: In a preliminary investigation (Susskind, M., Muller-Loennies, S., Nimmich, W., Brade, H., and Holst, O. (1995) Carbohydr. Res. 269, C1-C7), we identified after deacylation of lipopolysaccharides (LPS) from Klebsiella pneumoniae ssp. pneumoniae rough strain R20 (O1-:K20-) as a major fraction the oligosaccharide, where Kdo was 3-deoxy-D-manno-oct-2-ulopyranosonic acid and Hepp was manno-heptopyranose. The presence of the threo-hex-4-enuronopyranosyl residue indicated a substituent at O-4 of the second GalA residue linked to O-3 of the second L,D-Hep residue, which had been eliminated by treatment with hot alkali. We now report the complete structure of lipopolysaccharide, which was elucidated by additional characterization of isolated core oligosaccharides and analysis of the lipid A. The substituent at O-4 of the second GalpA is D-GlcpN, which in a fraction of the LPS is substituted at O-6 by three or four residues of D-glycero-D-manno-heptopyranose (D,D-Hepp). The complete carbohydrate backbone of the LPS is as follows, (L-glycero-D-manno-heptopyranose; L,D-Hepp), where all hexoses possess the D-configuration. Sugars marked with an asterisk are present in nonstoichiometric amounts. The structure is unique with regard to the presence of an alpha1fwdarw2-linked D-glycero-D-manno-heptoglycan (oligosaccharide), which has not been described to date, and does not contain phosphate substituents in the core region. Fatty acid analysis of lipid A identified (R)-3-hydroxytetradecanoic acid as sole amide-linked fatty acid and (R)-3-hydroxytetradecanoic acid, tetradecanoic acid, small amounts of 2-hydroxytetradecanoic acid, hexadecanoic acid, and traces of dodecanoic acid as ester-linked fatty acids, substituting the carbohydrate backbone D-GlcpN4Pbeta1fwdarw6D-GlcpNalpha1P. The nonreducing GlcN carries four fatty acids, present as two 3-O-tetradecanoyltetradecanoic acid residues, one of which is amide-linked and the other ester-linked to O-3'. The reducing GlcN is substituted in a nature fraction of lipid A by two residues of (R)-3-hydroxytetradecanoic acid, one in amide and the other in ester linkage at O-3. Two minor fractions of lipid A were identified; in one, the amide-linked (R)-3-hydroxytetradecanoic acid at the reducing GlcN is esterified with hexadecanoic acid, resulting in 3-O-hexadecanoyltetradecanoic acid, and in the second, one of the 3-O-tetradecanoyltetradecanoic acid residues at the nonreducing GlcN is %%%replaced%%% by 3-O-dodecanoyltetradecanoic acid. Thus, the complete structure of LPS is as shown in Fig. 1. After immunization of BALB/c mice, two monoclonal antibodies were obtained that were shown to be specific for the core of LPS from K. pneumoniae ssp. pneumoniae, since they did not react with LPS or whole-cell lysates of a variety of other

Gram-negative species. Both monoclonal antibodies could be inhibited by LPS but not by isolated oligosaccharides and are thus considered to recognize a conformational %%%epitope%%% in the core region.

9/7/10
DIALOG(R)File 5:Biosis Previews(R)

0011321782 BIOSIS NO.: 199800116029
Binding of single substituted promiscuous and designer peptides to purified DRB1*0101
AUTHOR: Macklin Kevin D (Reprint); Conti-Fine Bianca M
AUTHOR ADDRESS: Univ. Minnesota, Dep. Biochem., 1479 Gortner Ave., St. Paul, MN 55108, USA**USA
JOURNAL: Biochemical and Biophysical Research Communications 242 (2): p 322-326 Jan. 14, 1998 1998
MEDIUM: print
ISSN: 0006-291X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: MHC class II molecules present antigenic peptides to T cells. The sequence characteristics of peptides associated with various class H alleles have been examined by analysis of peptide mixtures extracted from purified class II molecules as well by direct binding assays with substituted synthetic peptides and purified class II molecules. Here, in vitro binding assays with purified DRBI*0101 and glycine substituted analogues of Hgamma321-340 and alanine substituted analogues of TT948-967, universal CD4+ epitopes of the gamma subunit of the human nicotinic acetylcholine receptor and tetanus %%%toxin%%%, respectively, were able to compete for binding to an extent similar to that of the unsubstituted peptides. Testing whether this is a property of promiscuous, but not allele-specific peptide epitopes, a designer peptide containing the proposed anchor residues for binding DRB1*0101 was used in similar binding assays. As expected, the binding capacity of the universal %%%epitope%%% peptides. However, substitution of the anchor residues for alanine in the context of this designer peptide did not abrogate binding to pears that for both class II allele-specific and universal %%%epitope%%% peptides, binding is a result of the combinatory effects of a few residues. The individual %%%replacement%%% of these residues with the sterically and electrostatically neutral residue alanine does not negatively affect binding in the continued presence of other anchor residues.

9/7/11
DIALOG(R)File 5:Biosis Previews(R)

0011254870 BIOSIS NO.: 199800049117
The carboxy-terminal C2-like domain of the alpha-%%%toxin%%% from Clostridium perfringens mediates calcium-dependent membrane recognition
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AUTHOR ADDRESS: Unite Genet. Mol. Bacterienne, Inst. Pasteur, 28 rue Docteur Roux, 75724 Paris Cedex 15, France**France
JOURNAL: Molecular Microbiology 26 (5): p867-876 Dec., 1997 1997
MEDIUM: print
ISSN: 0950-382X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The lethal, cytolytic alpha-%%%toxin%%% (phospholipase C) of Clostridium perfringens consists of two distinct modules: the larger N-terminal domain catalyses phospholipid hydrolysis, and its activity is potentiated by a smaller C-terminal domain. Calcium ions are essential for the binding of alpha-%%%toxin%%% to lipid films. Sixteen alpha-%%%toxin%%% variants with single amino acid substitutions in the

C-terminal region were obtained using site-directed mutagenesis and T7 expression technology. Five of these variants showed reduced phospholipase C activity and were considerably less active than native alpha-%%%toxin%%% under calcium-limiting conditions. %%%Replacement%%% of Thr-272 by Pro diminished phospholipase C activity, severely affected haemolysis and platelet aggregation and perturbed a surface-exposed conformational %%%epitope%%%. The results of sequence comparisons and molecular modelling indicate that the C-terminal region probably belongs to the growing family of C2 beta-barrel domains, which are often involved in membrane interactions, and that the functionally important substitutions are clustered at one extremity of the domain. The combined findings suggest that the C-terminal region of alpha-%%%toxin%%% mediates interactions with membrane phospholipids in a calcium-dependent manner. Mutations to this domain may account for the natural lack of toxicity of the alpha-%%%toxin%%% homologue, phospholipase C of Clostridium bifermentans.

9/7/12
DIALOG(R)File 5:Biosis Previews(R)

0010750292 BIOSIS NO.: 199799384352
Construction of an %%%epitope%%% vector utilising the diphtheria %%%toxin%%% B-subunit
AUTHOR: Johnson Nicholas (Reprint); Pickett Mark A; Watt Peter J; Clarke Ian N; Heckels John E
AUTHOR ADDRESS: Dep. Immunol., St. Bartholomew's R. London Sch. Med. Dent., 38 Little Britain, London EC1A 7BE, UK**UK
JOURNAL: FEMS Microbiology Letters 146 (1): p91-96 1997 1997
ISSN: 0378-1097
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: An immunogenic loop within the diphtheria %%%toxin%%% has been deleted from the B-subunit by a modification of the inverse polymerase chain reaction (IPCR) and %%%replaced%%% by a unique restriction endonuclease site. An oligonucleotide encoding an identified %%%epitope%%% sequence from the major outer membrane protein of Neisseria meningitidis of similar size and structure to that deleted has been introduced into the restriction site. Expression of the resulting chimeric B-subunit from Escherichia coli yielded a protein that was recognised by a panel of antibodies specific for the meningococcal %%%epitope%%%. Initial immunisation data suggest that this protein could elicit an antibody response against both diphtheria %%%toxin%%% and meningococcal proteins.

9/7/13
DIALOG(R)File 5:Biosis Previews(R)

0010232977 BIOSIS NO.: 199698700810
Improved antitumor activity of a recombinant anti-Lewis-y immunotoxin not requiring proteolytic activation
AUTHOR: Kuan Chien-Tsun; Pastan Ira (Reprint)
AUTHOR ADDRESS: Lab. Molecular Biol., Div. Basic Sci., Natl. Cancer Inst., Natl. Inst. Health, Build. 37, Room 4E16, 37 Convent Drive, MSC 4255, Bethesda, MD 20892-4255, USA**USA
JOURNAL: Proceedings of the National Academy of Sciences of the United States of America 93 (3): p974-978 1996 1996
ISSN: 0027-8424
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: B1(dsFv)-PE33 is a recombinant immunotoxin composed of a mutant form of Pseudomonas exotoxin (PE) that does not need proteolytic activation and a disulfide-stabilized Fv fragment of the anti-Lewis-y monoclonal antibody BI, which recognizes a carbohydrate %%%epitope%%% on

human carcinoma cells. In this molecule, amino acids 1-279 of PE are deleted and domain 1b (amino acids 365-394) is %%%replaced%%% by the heavy chain variable region (V-H) domain of monoclonal antibody B1. The light chain (V-L) domain is connected to the V-H domain by a disulfide bond. This recombinant %%%toxin%%%, termed B1(dsFv)-PE33, does not require proteolytic activation and it is smaller than other immunotoxins directed at Lewis-y, all of which require proteolytic activation. Furthermore, it is more cytotoxic to antigen-positive cell lines. B1(dsFv)-PE38 has the highest antitumor activity of anti-Lewis-y immunotoxins previously constructed. B1(dsFv)-PE33 caused complete regression of tumors when given at 12 mu-g/kg (200 pmol/kg) every other day for three doses, whereas B1(dsFv)-PE38 did not cause regressions at 13 mu-g/kg (200 pmol/kg). By bypassing the need for proteolytic activation and decreasing molecular size we have enlarged the therapeutic window for the treatment of human cancers growing in mice, so that complete remissions are observed at 2.5% of the LD-50.

9/7/14
DIALOG(R)File 5:Biosis Previews(R)

0009948350 BIOSIS NO.: 199598416183
Induction of %%%toxin%%% sensitivity in insect cells by infection with baculovirus encoding diphtheria %%%toxin%%% receptor
AUTHOR: Valdizan Elsa M; Loukianov Evgenij V; Olsnes Sjur (Reprint)
AUTHOR ADDRESS: Biochem. Dep., Inst. Cancer Res., Norwegian Radium Hosp., Montebello, 0310 Oslo, Norway**Norway
JOURNAL: Journal of Biological Chemistry 270 (28): p16879-16885 1995 1995
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The diphtheria %%%toxin%%% receptor (DTR) has been identified as the precursor of heparin-binding epidermal growth factor-like growth factor, which may interact with other membrane proteins to form the functional receptor. To test if mammalian DTR is able to confer %%%toxin%%% sensitivity onto phylogenetically distant cells, we expressed monkey DTR in the baculovirus system and tested infected insect cells for %%%toxin%%% sensitivity. cDNA encoding an %%%epitope%%%-tagged heparin-binding epidermal growth factor-like growth factor precursor (DTR-B3) was inserted into the virus genome by allelic %%%replacement%%% to construct the recombinant virus vAc-DTR-B3. SF9 cells infected with vAc-DTR-B3, expressed functional DTR, which could be precipitated from the solubilized membrane fraction of infected cells with Sepharose-immobilized diphtheria %%%toxin%%%. The highest level of expression (about 5 times 10-6 receptors/cell) was observed 48 h after infection, at which time the infected cells were highly sensitive to diphtheria %%%toxin%%%. Uninfected SF9 cells and cells infected with the wild type virus were resistant to the %%%toxin%%%. The presence of heparin increased both the binding and the %%%toxin%%% sensitivity of vAc-DTR-B3-infected SF9 cells. Translocation of %%%toxin%%% A fragment was induced when cells with surface-bound %%%toxin%%% were exposed to low pH, and the translocation was optimal at pH ltoreq 5.5. It was apprx 100 times more efficient at 24 degree C than at 4 degree C. The data indicate that monkey DTR is fully functional when expressed in insect cells.

9/7/15
DIALOG(R)File 5:Biosis Previews(R)

0009913900 BIOSIS NO.: 199598381733
Immunogenicity of T %%%epitope%%%-containing cyclic peptides: Increasing neutralizing antibody responses by introducing fine chemical changes
AUTHOR: Leonetti Michel; Cotton Joel; Leroy Sandrine; Mourier Gilles; Menez Andre
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JOURNAL: Journal of Immunology 155 (1): p210-218 1995 1995

ISSN: 0022-1767
DOCUMENT TYPE: Article
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LANGUAGE: English

ABSTRACT: We showed previously that the disulfide-containing T peptide 24-41C from a highly structured snake %%%toxin%%% elicits, in a free state, Abs that neutralize the %%%toxin%%%, and only a turn structure commonly exists in 24-41C and the corresponding %%%toxin%%% region. To tentatively increase the neutralizing capacity of antipeptide Abs, we 1) %%%replaced%%% Gly-40 by an aminoisobutyric moiety (24-41 Aib), 2) substituted the half cystines 24 and 41 by penicillamine moieties (24-41 Pen), and 3) introduced an amide bond between the epsilon-NH2 of Lys-27 and the gamma-COOH of Glu-3B (24-41 K-E). A solution ELISA made with antitoxin Abs revealed that 24-41 Pen is more antigenic than 24-41 Aib and 24-41 C, which are more antigenic than 24-41 K-E, suggesting that the conformation of 24-41 Pen is most closely related to the corresponding region in the native %%%toxin%%%. The peptides 24-41 Pen, 24-41 Aib, and 24-41C stimulate T cells from BALB/c mice, whereas 24-41 K-E has lost this property and thereby fails to elicit Abs. Finally, anti-24-41 Pen Abs are more potent at neutralizing the native %%%toxin%%% than anti-24-41C Abs, which are more potent than anti-24-41 Aib Abs. The efficacy of anti-24-41 Pen Abs was similar to that of a %%%toxin%%% specific mAb. Therefore, introduction of appropriate constraints makes it possible to improve the neutralizing Ab response raised by a synthetic peptide. Such observations should be of interest for the design of efficient synthetic vaccines.

9/7/16
DIALOG(R)File 5:Biosis Previews(R)

0008745601 BIOSIS NO.: 199395047867
Activation of the alpha subunit of G-s in intact cells alters its abundance, rate of degradation, and membrane avidity
AUTHOR: Levis Mark J (Reprint); Bourne Henry R
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JOURNAL: Journal of Cell Biology 119 (5): p1297-1307 1992
ISSN: 0021-9525
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Binding of GTP induces alpha subunits of heterotrimeric G proteins to take on an active conformation, capable of regulating effector molecules. We expressed %%%epitope%%%-tagged versions of the alpha subunit (alpha-S) of G-S in genetically alpha-S-deficient S49 cyc- cells. Addition of a hemagglutinin (HA) %%%epitope%%% did not alter the ability of wild type alpha-S to mediate hormonal stimulation of adenylyl cyclase or to attach to cell membranes. The HA %%%epitope%%% did, however, allow a mAb to immunoprecipitate the recombinant protein (HA-alpha-S) quantitatively from cell extracts. We activated the %%%epitope%%%-tagged alpha-S in intact cells by: (a) exposure of cells to cholera %%%toxin%%%, which activates alpha-S by covalent modification; (b) mutational %%%replacement%%% of arginine-201 in HA-alpha-S by a cysteine residue, to create HA-alpha-S-R201C; like the cholera %%%toxin%%%-catalyzed modification, this mutation activates alpha-S by slowing its intrinsic GTPase activity; and (c) treatment of cells with the beta-adrenoceptor agonist, isoproterenol, which promotes binding of GTP to alpha-S, thereby activating adenylyl cyclase. Both cholera %%%toxin%%% and the R201C mutation accelerated the rate of degradation of alpha-S (0.03 h-1) by three-to fourfold and induced a partial shift of the protein from a membrane bound to a soluble compartment. At steady state, 80% of HA-alpha-S-R201C was found in the soluble fraction, as compared to 10% of wild type HA-alpha-S. Isoproterenol rapidly (in lt 2 min) caused 20% of HA-alpha-S to shift from the membrane-bound to the soluble compartment. Cholera %%%toxin%%% induced a 3.5-fold increase in the rate of degradation of a second mutant, HA-alpha-S-G226A, but did not cause it to move into the soluble fraction; this observation shows that

loss of membrane attachment is not responsible for the accelerated degradation of alpha-S in response to activation. Taken together, these findings show that activation of alpha-S induces a conformational change that loosens its attachment to membranes and increases its degradation rate.

9/7/17
DIALOG(R)File 5:Biosis Previews(R)

0008423034 BIOSIS NO.: 199294124875
PSEUDOMONAS-AERUGINOSA EXOTOXIN A INTERACTION WITH EUCARYOTIC ELONGATION FACTOR 2 ROLE OF THE HIS-426 RESIDUE
AUTHOR: KESSLER S P (Reprint); GALLOWAY D R
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JOURNAL: Journal of Biological Chemistry 267 (27): p19107-19111 1992
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Pseudomonas aeruginosa exotoxin A (ETA) catalzyes the transfer of the ADP-ribose moiety of NAD+ onto eucaryotic elongation factor 2 (EF-2). To study the ETA site of interaction with EF-2, an immobilized EF-2 binding assay was developed. This assay demonstrates that ETA, in the presence of NAD+, binds to immobilized EF-2. Additionally, diphtheria %%%toxin%%% was also found to bind to the immobilized EF-2 in the presence of NAD+. Comparative analysis was performed with a mutated form of ETA (CRM 66) in which a histidine residue at position 426 has been %%%replaced%%% with a tyrosine residue. This immunologically cross-reactive, ADP-ribosyl transferase-deficient %%%toxin%%% does not bind to immobilized EF-2, thus explaining its lack of ADPRT activity. ETA bound to immunobilized EF-2 cannot bind the monoclonal antibody TC-1 which specifically recognizes the ETA %%%epitope%%% containing His426. Immunoprecipitation of native ETA by mAb TC-1 is only achieved by incubating ETA in the presence of NAD+. Diethyl pyrocarbonate modification of the His426 residue blocks ETA binding to EF-2 and prevents the binding of the TC-1 antibody. Analogs of NAD+ containing a reduced nicotinamide ring of modified adenine moieties cannot substitute for NAD+ in the immobilized binding assay. Collectively, these data support our proposal that the site of ETA interaction with EF-2 includes His426 and that a molecule of NAD+ is required for stable interaction.

9/7/18
DIALOG(R)File 5:Biosis Previews(R)

0008162563 BIOSIS NO.: 199293005454
ROLE OF SINGLE AMINO ACIDS IN THE RECOGNITION OF A T CELL %%%EPITOPE%%%
AUTHOR: SUHRBIER A (Reprint); RODDA S J; HO P C; CSURHES P; DUNCKLEY H; SAUL A; GEYSEN H M; RZEPCZYK C M
AUTHOR ADDRESS: QUEENSLAND INSTITUTE MEDICAL RESEARCH, BRAMSTON TERRACE, BRISBANE, QUEENSLAND 4006, AUST**AUSTRALIA
JOURNAL: Journal of Immunology 147 (8): p2507-2513 1991
ISSN: 0022-1767
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: T cell epitopes can be defined by the use of synthetic peptides, which when added to APC efficiently mimic naturally processed Ag. Free peptide is thought to bind to cell-surface MHC glycoproteins and the TCR then recognizes the resulting complex. The specificity of a tetanus %%%toxin%%%-specific human Th cell clone was investigated using a complete %%%replacement%%% set of peptides in which every amino acid within the minimal T cell %%%epitope%%% was %%%replaced%%% by each of the 19 alternative genetically coded amino acids. Within the minimal %%%epitope%%%, found to be YSYFPSVI (tetanus %%%toxin%%% 593-600), a

small number of substitutions could be made without significant loss of activity, defined as substitutions giving peptides whose activity fell within .+-.3 SD of the mean parent response. Y593 could be substituted with F, W, M, L, V, and I; S594 with G and T; Y595, F596, and P597 with no other amino acids; S598 with A; V599 with S, and I600 with L. Rank ordering of the substitutions allowed a precise description to be made of MHC and/or TCR interaction with each amino acid side chain within the %%%epitope%%%. Simplified theoretic calculations based on this study indicate that class II T cell recognition has a specificity greater than 1 in 108. Competition experiments indicate that Y595, F596, P597, and I600 are critical for binding of this %%%epitope%%% to its restricting element, HLA DR4Dw14.

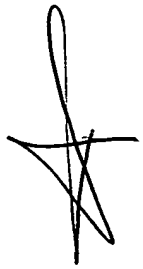
9/7/19
DIALOG(R)File 5:Biosis Previews(R)

0007634346 BIOSIS NO.: 199191017237
HIGH-LEVEL PRODUCTION OF ESCHERICHIA-COLI STB HEAT-STABLE ENTEROTOXIN AND QUANTIFICATION BY A DIRECT ELISA
AUTHOR: URBAN R G (Reprint); PIPPER E M; DREYFUS L A; WHIPP S C
AUTHOR ADDRESS: DEP MICROBIOLOGY, UNIVERSITY TEXAS MEDICAL BRANCH, GALVESTON, TEX 77550, USA**USA
JOURNAL: Journal of Clinical Microbiology 28 (11): p2383-2388 1990
ISSN: 0095-1137
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: A convenient and sensitive enzyme-linked immunosorbent assay (ELISA) for the STb heat-stable enterotoxin of Escherichia coli was developed and used to quantify STb production by strains with a high level of expression. Based on an antigenic profile of the secreted form of STb, a synthetic peptide (STb3-27) spanning the major predicted %%%epitope%%% was synthesized, coupled to keyhole limpet hemocyanin, and used to immunize rabbits. Anti-STb3-27 antibodies were affinity purified on a synthetic peptide-Sepharose 4B column and used in a direct-binding STb ELISA. Based on a highly purified form of %%%toxin%%% as a standard, the ELISA detected as little as 1 to 2 ng of STb from crude culture filtrates. ELISA data revealed that natural STb-producing strains elaborate little STb in defined-medium cultures relative to that elaborated by a recombinant strain harboring a cloned copy of the estB gene. %%%Replacement%%% of the endogenous STb promoter with any of several highly active promoters, including a bacteriophage T7 promoter, a .beta.-galactosidase promoter, and a tryptophan-.beta.galactosidase hybrid (tac) promoter, increased the yield of STb 10- to 20-fold over levels obtained by an E. coli strain harboring the recombinant estB gene. The high level of STb antigen detected by the ELISA correlated with intestinal secretory activity. The combination of a convenient assay and effective hyperproduction of STb will serve as a basis for a large-scale %%%toxin%%% purification strategy.

9/7/20
DIALOG(R)File 5:Biosis Previews(R)

0007326837 BIOSIS NO.: 199090111316
MUTANTS OF STAPHYLOCOCCAL TOXIC SHOCK SYNDROME %%%TOXIN%%% 1 MITOGENICITY AND RECOGNITION BY A NEUTRALIZING MONOCLONAL ANTIBODY
AUTHOR: BLANCO L (Reprint); CHOI E M; CONNOLLY K; THOMPSON M R; BONVENTRE P F
AUTHOR ADDRESS: DEP MOL GEN, BIOCHEM AND MICROBIOL, UNIV CINCINNATI MED CENT, CINCINNATI, OHIO 45267-0524, USA**USA
JOURNAL: Infection and Immunity 58 (9): p3020-3028 1990
ISSN: 0019-9567
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH



ABSTRACT: Toxic shock syndrome %%%toxin%%% 1 (TSST-1) a 22-kilodalton protein made by strains of Staphylococcus aureus harboring the chromosomal %%%toxin%%% gene, may elicit toxic shock syndrome in humans. In vitro, TSST-1 induces T cells to proliferate and macrophages to secrete interleukin-1. To conduct a structure-function analysis, point mutations on the TSST-1 gene were generated by site-directed mutagenesis to identify amino acids critical for activity of the %%%toxin%%%. Specific tyrosine and histidine residues were %%%replaced%%% by alanines. Wild-type and mutant TSST-1 gene constructs were expressed in Escherichia coli, and the products were tested for their mitogenic potential and reactivity with a TSST-1 neutralizing monoclonal antibody (MAb 8-5-7). Four of the mutants were similar to the wild type; i.e., the mutant toxins stimulated murine T cells reacted with MAb 8-5-7 equally as well as the wild type. Two mutants exhibited a decrease in mitogenic activity, but one of these retained the capacity to bind with MAb 8-5-7 while the other was no longer recognized by the same antibody. One double mutant demonstrated minimal mitogenic activity and did not react in enzyme-linked immunosorbent and immunoblot assays with MAb 8-5-7. The data show that specific residues near the carboxy terminus of TSST-1 are essential for mitogenic activity and in forming the %%%epitope%%% recognized by neutralizing MAb 8-5-7.

9/7/21
DIALOG(R)File 5:Biosis Previews(R)

0007200956 BIOSIS NO.: 199089118847
POLYVALENT SYNTHETIC VACCINES RELATIONSHIP BETWEEN T EPITOPES AND IMMUNOGENICITY
AUTHOR: JOLIVET M (Reprint); LISE L; GRAS-MASSE H; TARTAR A; AUDIBERT F; CHEDID L
AUTHOR ADDRESS: BIOMERIEUX MARCY ETOILE, 69752 CHARBONNIERES LES BAINS, FR **FRANCE
JOURNAL: Vaccine 8 (1): p35-40 1990
ISSN: 0264-410X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: Three different synthetic polyvalent vaccines have been constructed by conjugating four synthetic peptides without any carrier protein. The peptides were copy fragments of two bacterial antigens (Streptococcus pyogenes M protein and diphtheria %%%toxin%%%), two parasitic antigens (circumsporozoite protein of Plasmodium falciparum and Plasmodium knowlesi), and one viral antigen (hepatitis B surface antigen). Outbred guinea-pigs immunized with polyvalent vaccine containing streptococcal, diphtheric, P. knowlesi and hepatitis peptides raised high specific antibody response against the four specificities. Individual T cell analysis demonstrated that hepatitis peptide bears a T dominant %%%epitope%%%. A similar immune response was obtained with a second polyvalent vaccine where the P. knowlesi peptide had been %%%replaced%%% by the P. falciparum peptide. In both experiments the malarial peptides behave like pure B epitopes. Prediction of immunodominant helper T-cell antigenic sites were performed with the five peptides using computer algorithm. Hepatitis and diphtheric peptides were selected whereas the streptococcal peptide was rejected although it can experimentally contain a T %%%epitope%%%. To confirm this result animals were immunized with a third polyvalent vaccine which does not contain the hepatitis peptide. No T cell proliferation or antipeptide antibodies were detected. These results demonstrate that the cooperative immune response requires a certain degree of antigenic complexity for the induction of antibody response.
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$246.38 Estimated cost File5
$4.26 TELNET

$250.64 Estimated cost this search
$250.72 Estimated total session cost 5.469 DialUnits
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File 5:Biosis Previews(R) 1969-2005/Jul W4
(c) 2005 BIOSIS

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Set Items Description
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Set	Items	Description
S1	20	GELONIN AND FUSION
S2	9	GELONIN AND FRAGMENT?
S3	0	GELONIN AND SFVMEL
S4	64	AU='ROSENBLUM MICHAEL G' OR AU='ROSENBLUM MICHEAL G'
S5	33	E3-E5
S6	21	S4 AND GELONIN
S7	13	S5 AND GELONIN
S8	21	S6 OR S7

? t s1/7/1-20

1/7/1
DIALOG(R)File 5:Biosis Previews(R)
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0015362336 BIOSIS NO.: 200510056836
Targeting skeletal metastases in prostate cancer with a novel VEGF121 %%%fusion%%% toxin
AUTHOR: Poblenz A T (Reprint); Mohamedali K; Rosenblum M G; Darnay B G
AUTHOR ADDRESS: Univ Texas, MD Anderson Canc Ctr, Houston, TX 77030 USA** USA
JOURNAL: Journal of Bone and Mineral Research 19 pS67 OCT 04 2004
CONFERENCE/MEETING: 26th Annual Meeting of the American-Society-for-Bone-and-Mineral-Research Seattle, WA, USA October 01 -05, 2004; 20041001
SPONSOR: Amer Soc Bone & Mineral Res
ISSN: 0884-0431
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

1/7/2
DIALOG(R)File 5:Biosis Previews(R)
(c) 2005 BIOSIS. All rts. reserv.

0015124779 BIOSIS NO.: 200500031844
Intein-mediated %%%fusion%%% expression, high efficient refolding, and one-step purification of %%%gelonin%%% toxin
AUTHOR: Guo Chenyun; Li Zhuoyu; Shi Yawei; Xu Mingqun; Wise John G; Trommer Wolfgong E; Yuan Jingming (Reprint)
AUTHOR ADDRESS: Inst BiotechnolMinist EducKey Lab Chem Biol and Mol Engn, Shanxi Agr Univ, Taiyuan, 030006, China**China
AUTHOR E-MAIL ADDRESS: jmyuan@sxu.edu.cn
JOURNAL: Protein Expression and Purification 37 (2): p361-367 October 2004 2004
MEDIUM: print
ISSN: 1046-5928
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: An open reading frame of %%%gelonin%%% (Gel), one of ribosome inactivating proteins, was inserted into the vector pBSL-C which contains the coding region of chitin binding domain (CBD)-intein, resulting in the %%%fusion%%% expression of CBD-intein-Gel in Escherichia coli BL21 (DE3) by the induction of IPTG. The %%%fusion%%% product formed an aggregate of the misfolded protein, commonly referred to as inclusion bodies (IBs). The IBs were denatured and then refolded by step-wise dialysis. About 69% %%%fusion%%% protein was in vitro refolded to native state in the presence of GSSG and GSH as monitored by size exclusion HPLC. The refolded CBD-intein-Gel was loaded onto chitin beads column equilibrated with 10 mM Tris buffer, 500 mM NaCl, pH 8.5, and about 2.4 mg Gel/L culture with 96% homogeneity was directly eluted from the captured column

by incubation at 25degreeC under pH 6.5 for 48 h based on intein C-terminal self-cleavage. Western blot, ELISA, and in vitro inhibition of protein synthesis demonstrated that the bioactivity of recombinant Gel was comparable to that of native Gel purified from seeds. This implied that the purified Gel by this method is biologically active and suitable for further studies.

1/7/3
DIALOG(R)File 5:Biosis Previews(R)

0014492556 BIOSIS NO.: 200300451235
In vitro and in vivo anti-angiogenic effects of VEGF121/rGel %%%fusion%%% toxin.
AUTHOR: Mohamedali Khalid A (Reprint); Kedar Daniel; Ran Sophia; Garcia Bobby; Dinney Colin P; Thorpe Philip; Rosenblum Michael G
AUTHOR ADDRESS: UT M. D. Anderson Cancer Center, Houston, TX, USA**USA
JOURNAL: Proceedings of the American Association for Cancer Research Annual Meeting 44 p7 July 2003 2003
MEDIUM: print
CONFERENCE/MEETING: 94th Annual Meeting of the American Association for Cancer Research Washington, DC, USA July 11-14, 2003; 20030711
ISSN: 0197-016X
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

1/7/4
DIALOG(R)File 5:Biosis Previews(R)

0014492534 BIOSIS NO.: 200300451213
VEGF121 %%%gelonin%%% %%%fusion%%% protein inhibits breast cancer metastasis in nude mice.
AUTHOR: Ran Sophia (Reprint); Mohamedali Khalid; Thorpe Philip E; Rosenblum Michael G
AUTHOR ADDRESS: University of Texas at Dallas, Dallas, TX, USA**USA
JOURNAL: Proceedings of the American Association for Cancer Research Annual Meeting 44 p1 July 2003 2003
MEDIUM: print
CONFERENCE/MEETING: 94th Annual Meeting of the American Association for Cancer Research Washington, DC, USA July 11-14, 2003; 20030711
ISSN: 0197-016X
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

1/7/5
DIALOG(R)File 5:Biosis Previews(R)

0014421889 BIOSIS NO.: 200300379166
Design, expression, purification, and characterization, in vitro and in vivo, of an antimelanoma single-chain Fv antibody fused to the toxin %%%gelonin%%%.
AUTHOR: Rosenblum Michael G (Reprint); Cheung Lawrence H; Liu Yuying; Marks John W
AUTHOR ADDRESS: Section of Immunopharmacology and Targeted Therapy, Department of Bioimmunotherapy, University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Unit 44, Houston, TX, 77030, USA**USA
AUTHOR E-MAIL ADDRESS: mrosenbl@notes.mdacc.tmc.edu
JOURNAL: Cancer Research 63 (14): p3995-4002 July 15, 2003 2003
MEDIUM: print
ISSN: 0008-5472 _(ISSN print)
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: We constructed a single-chain anti-gp240 antibody (designated MEL sFv) and fused this to the recombinant toxin %%%gelonin%%% (rGel). MEL sFv-rGel was produced in bacterial expression plasmid (pET-32), and the protein composition was confirmed by both DNA sequencing and Western analysis. Inhibition of cell-free protein synthesis by the %%%fusion%%% construct demonstrated an IC50 of 100 pM, comparable with that for native %%%gelonin%%% (104 pM). The MEL sFv-rGel %%%fusion%%% toxin bound to antigen-positive but not antigen-negative cells as assessed by ELISA. Internalization into A-375 target cells was demonstrable by 1 h after exposure. Against A-375 cells, MEL sFv-rGel demonstrated an IC50 of approximately 8 nM, which was 250-fold lower than that for free rGel (2000 nM). The cytotoxic effects of the construct did not involve apoptosis because terminal deoxynucleotidyl transferase-mediated nick end labeling assays of treated cells were negative. 125I-labeled MEL sFv-rGel demonstrated biphasic clearance of the construct from plasma (t1/2 alpha and t1/2 beta were 0.46 and 7.2 h, respectively). At 72 h after administration, xenograft studies showed that the tissue: blood ratio was highest for tumor followed by spleen, kidney, and liver. Groups of tumor-bearing nude mice were treated with %%%fusion%%% toxin at either 2 or 20 mg/kg. Compared with saline-treated controls, for which mean tumor burden increased 6-fold, the groups treated with the high and low doses of %%%fusion%%% construct showed no increase or only a 2-fold increase, respectively. These studies suggest that this recombinant %%%fusion%%% construct has potent cytotoxic activity both in vitro and in vivo and is an excellent candidate for clinical development.

1/7/6
DIALOG(R)File 5:Biosis Previews(R)

0014077598 BIOSIS NO.: 200300036317
Diphtheria %%%fusion%%% protein therapy of chemoresistant malignancies.
AUTHOR: Frankel Arthur E (Reprint); Rossi Patrick; Kuzel Timothy M; Foss Francine
AUTHOR ADDRESS: Wake Forest University School of Medicine, Medical Center Drive, Hanes 4046, Winston-Salem, NC, 27157, USA**USA
AUTHOR E-MAIL ADDRESS: afrankel@wfubmc.edu
JOURNAL: Current Cancer Drug Targets 2 (1): p19-36 March 2002 2002
MEDIUM: print
ISSN: 1568-0096 _(ISSN print)
DOCUMENT TYPE: Article; Literature Review
RECORD TYPE: Citation
LANGUAGE: English

1/7/7
DIALOG(R)File 5:Biosis Previews(R)

0013850409 BIOSIS NO.: 200200443920
Targeting bladder tumor vascular endothelium with VEGF/rGel %%%fusion%%% toxin
AUTHOR: Kedar Daniel (Reprint); Sweeney Paul (Reprint); Huang Samuel (Reprint); Mian Badar (Reprint); Rosenblum Michael G (Reprint); Dinney Colin P (Reprint)
AUTHOR ADDRESS: Houston, TX, USA**USA
JOURNAL: Journal of Urology 167 (4 Supplement): p120 April, 2002 2002
MEDIUM: print
CONFERENCE/MEETING: Annual Meeting of the American Urology Association, Inc. Orlando, Florida, USA May 25-30, 2002; 20020525
ISSN: 0022-5347
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

1/7/8
DIALOG(R)File 5:Biosis Previews(R)

0013825671 BIOSIS NO.: 200200419182
VEGF121-%%%gelonin%%% %%%fusion%%% toxin specifically localizes to tumor blood vessels and induces robust vascular damage in human tumor xenograft models
AUTHOR: Ran Sophia (Reprint); Mohamedali Khalid; Cheung Lawrence; Thorpe Philip E; Rosenblum Michael G
AUTHOR ADDRESS: Southwestern Medical Center, UT at Dallas, Dallas, TX, USA **USA
JOURNAL: Proceedings of the American Association for Cancer Research Annual Meeting 43 p1006 March, 2002 2002
MEDIUM: print
CONFERENCE/MEETING: 93rd Annual Meeting of the American Association for Cancer Research San Francisco, California, USA April 06-10, 2002; 20020406
ISSN: 0197-016X
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

1/7/9
DIALOG(R)File 5:Biosis Previews(R)

0013808858 BIOSIS NO.: 200200402869
In vitro and in vivo studies of a VEGF121/rGelonin chimeric %%%fusion%%% toxin targeting the neovasculature of solid tumors
AUTHOR: Veenendaal Liesbeth M; Jin Hangqing; Ran Sophia; Cheung Lawrence; Navone Nora; Marks John W; Waltenberger Johannes; Thorpe Philip; Rosenblum Michael G (Reprint)
AUTHOR ADDRESS: Immunopharmacology and Targeted Therapy Section, Department of Bioimmunotherapy, M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Box 44, Houston, TX, 77030-4009, USA**USA
JOURNAL: Proceedings of the National Academy of Sciences of the United States of America 99 (12): p7866-7871 June 11, 2002 2002
MEDIUM: print
ISSN: 0027-8424
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Vascular endothelial growth factor (VEGF) plays a key role in the growth and metastasis of solid tumors. We generated a %%%fusion%%% protein containing VEGF121 linked by a flexible G4S tether to the toxin %%%gelonin%%% (rGel) and expressed this as a soluble protein in bacteria. Purified VEGF121/rGel migrated as an 84-kDa homodimer under non-reducing conditions. VEGF121/rGel bound to purified, immobilized Flk-1, and the binding was competed by VEGF121. Both VEGF121/rGel and VEGF121 stimulated cellular kinase insert domain receptor (KDR) phosphorylation. The VEGF121/rGel %%%fusion%%% construct was highly cytotoxic to endothelial cells overexpressing the KDR/Flk-1 receptor. The IC50 of the construct on dividing endothelial cells expressing 105 or more KDR/Flk-1 receptors per cell was 0.5-1 nM, as compared with 300 nM for rGel itself. Dividing endothelial cells overexpressing KDR were approximately 60-fold more sensitive to VEGF121/rGel than were nondividing cells. Endothelial cells overexpressing FLT-1 were not sensitive to the %%%fusion%%% protein. Human melanoma (A-375) or human prostate (PC-3) xenografts treated with the %%%fusion%%% construct demonstrated a reduction in tumor volume to 16% of untreated controls. The %%%fusion%%% construct localized selectively to PC-3 tumor vessels and caused thrombotic damage to tumor vessels with extravasation of red blood cells into the tumor bed. These studies demonstrate the successful use of VEGF121/rGel %%%fusion%%% construct for the targeted destruction of tumor vasculature in vivo.

1/7/10
DIALOG(R)File 5:Biosis Previews(R)

0013515250 BIOSIS NO.: 200200108761
Immunotoxins comprising ribosome-inactivating proteins

AUTHOR: Better M D; Carroll S F; Studnicka G M
AUTHOR ADDRESS: Los Angeles, Calif., USA**USA
JOURNAL: Official Gazette of the United States Patent and Trademark Office Patents 1210 (4): p3653 May 26, 1998 1998
MEDIUM: print
ISSN: 0098-1133
DOCUMENT TYPE: Patent
RECORD TYPE: Citation
LANGUAGE: English

1/7/11
DIALOG(R)File 5:Biosis Previews(R)
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0013444812 BIOSIS NO.: 200200038323
In vitro and in vivo studies of VEGF/rGel %%%fusion%%% toxin targeting tumor vascular endothelium
AUTHOR: Jin Hangqing (Reprint); Ran Sophia; Thorpe Philip; Rosenblum Michael G
AUTHOR ADDRESS: University of Texas, MD Anderson Cancer Center, Houston, TX, USA**USA
JOURNAL: Proceedings of the American Association for Cancer Research Annual Meeting 42 p822 March, 2001 2001
MEDIUM: print
CONFERENCE/MEETING: 92nd Annual Meeting of the American Association for Cancer Research New Orleans, LA, USA March 24-28, 2001; 20010324
ISSN: 0197-016X
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

1/7/12
DIALOG(R)File 5:Biosis Previews(R)
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0012520583 BIOSIS NO.: 200000238896
In vitro studies of a recombinant, single-chain immunotoxin recognizing the HER2/neu proto-oncogene and containing the toxin %%%gelonin%%%
AUTHOR: Marks John W (Reprint); Cheung Lawrence H (Reprint); Rosenblum Michael G (Reprint)
AUTHOR ADDRESS: M D Anderson Cancer Ctr, Houston, TX, USA**USA
JOURNAL: Proceedings of the American Association for Cancer Research Annual Meeting (41): p529-530 March, 2000 2000
MEDIUM: print
CONFERENCE/MEETING: 91st Annual Meeting of the American Association for Cancer Research. San Francisco, California, USA April 01-05, 2000; 20000401
ISSN: 0197-016X
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

1/7/13
DIALOG(R)File 5:Biosis Previews(R)
(c) 2005 BIOSIS. All rts. reserv.

0012520259 BIOSIS NO.: 200000238572
In vitro and in vivo studies of VEGF121/rGel %%%fusion%%% toxin targeting tumor neovasculature
AUTHOR: Cheung Lawrence (Reprint); Veenendaal Liesbeth; Marks John W; Ran Sophia; Thorpe Philip; Rosenblum Michael G
AUTHOR ADDRESS: M D Anderson Cancer Ctr, Houston, TX, USA**USA
JOURNAL: Proceedings of the American Association for Cancer Research Annual Meeting (41): p387 March, 2000 2000
MEDIUM: print
CONFERENCE/MEETING: 91st Annual Meeting of the American Association for Cancer Research. San Francisco, California, USA April 01-05, 2000; 20000401

ISSN: 0197-016X
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

1/7/14
DIALOG(R)File 5:Biosis Previews(R)

0010898604 BIOSIS NO.: 199799532664
A recombinant single-chain antibody %%%fusion%%% toxin selectively targets gp240-expressing human melanoma cells
AUTHOR: Parakh C R; Marks J W; Rosenblum M G
AUTHOR ADDRESS: Univ. Texas M.D. Anderson Cancer Center, Houston, TX 77096, USA**USA
JOURNAL: Proceedings of the American Association for Cancer Research Annual Meeting 38 (0): p487-488 1997 1997
CONFERENCE/MEETING: Eighty-eighth Annual Meeting of the American Association for Cancer Research San Diego, California, USA April 12-16, 1997; 19970412
ISSN: 0197-016X
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

1/7/15
DIALOG(R)File 5:Biosis Previews(R)

0010623110 BIOSIS NO.: 199699257170
Cell surface receptor directed targeting of toxin to human malaria parasite; Plasmodium falciparum
AUTHOR: Surolia Namita (Reprint); Misquith Sandra
AUTHOR ADDRESS: Jawaharlal Nehru Cent. Adv. Sci. Res., Jakkur, Bangalore 560 064, India**India
JOURNAL: FEBS Letters 396 (1): p57-61 1996 1996
ISSN: 0014-5793
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: %%%Gelonin%%% (a toxin and type II ribosome inactivating protein) when linked to human transferrin can be targeted to Plasmodium falciparum. The transferrin toxin conjugate is significantly toxic to parasite growth and is 25 times more potent than toxin alone in inhibiting parasite protein synthesis. The mechanism of its entry into the intraerythrocytic parasite is discussed.

1/7/16
DIALOG(R)File 5:Biosis Previews(R)

0009908895 BIOSIS NO.: 199598376728
T Cell-targeted Immunofusion Proteins from Escherichia coli
AUTHOR: Better Marc (Reprint); Bernhard Susan L; Williams Robert E; Leigh Scott D; Bauer Robert J; Kung Ada H C; Carroll Stephen F; Fishwild Dianne M
AUTHOR ADDRESS: Xoma Corp., 1545 17th St., Santa Monica, CA 90404, USA**USA
JOURNAL: Journal of Biological Chemistry 270 (25): p14951-14957 1995 1995
ISSN: 0021-9258
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: %%%Fusion%%% proteins between cell-targeting domains and cytotoxic proteins should be particularly effective therapeutic reagents. We constructed a family of immunofusion proteins linking humanized Fab, F(ab')-2, or single chain antibody forms of the H65 antibody (which

recognizes the CD5 antigen on the surface of human T cells) with the plant ribosome-inactivating protein %%%gelonin%%%. We reasoned that such an immunofusion would kill human target cells as efficiently as the previously described chemical conjugates of H65 and %%%gelonin%%% (Better M., Bernhard, S. L., Fishwild, D. M., Nolan, P. A., Bauer, R. J., Kung, A. H. C., and Carroll, S. F. (1994) J. Biol. Chem. 269, 9644-9650) if both the recognition and catalytic domains remained active, and a proper linkage between domains could be found. Immunofusion proteins were produced in Escherichia coli as secreted proteins and were recovered directly from the bacterial culture supernatant in an active form. All of the immunofusion proteins were purified by a common process and were tested for cytotoxicity toward antigen-positive human cells. A 20-60-fold range of cytotoxic activity was seen among the %%%fusion%%% family members, and several %%%fusion%%% proteins were identified which are approximately as active as effective chemical conjugates. Based on these constructs, immunofusion avidity and potency can be controlled by appropriate selection of antibody domains and ribosome-inactivating protein.

1/7/17
DIALOG(R)File 5:Biosis Previews(R)

0009733558 BIOSIS NO.: 199598201391
Cloning a single-chain analogue of the anti-melanoma monoclonal antibody ZME-018: Construction and testing of an antibody-recombinant %%%gelonin%%% %%%fusion%%% toxin
AUTHOR: Parakh Cushrow R; Cheung Lawrence; Rosenblum Michael G
AUTHOR ADDRESS: Univ. Tex. M. D. Anderson Cancer Cent., 1515 Holcombe Boulevard, Houston, TX 77030, USA**USA
JOURNAL: Proceedings of the American Association for Cancer Research Annual Meeting 36 (0): p488 1995 1995
CONFERENCE/MEETING: Eighty-sixth Annual Meeting of the American Association for Cancer Research Toronto, Ontario, Canada March 18-22, 1995; 19950318
ISSN: 0197-016X
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

1/7/18
DIALOG(R)File 5:Biosis Previews(R)

0009733557 BIOSIS NO.: 199598201390
Construction and testing of a recombinant single-chain antibody-%%%gelonin%%% %%%fusion%%% toxin directed against the cell surface growth factor receptor p185-HER-2/neu
AUTHOR: Parakh Cushrown R (Reprint); Cheung Lawrence (Reprint); King C Richter; Sastry-Dent Lakshmi; Rosenblum Michael G (Reprint)
AUTHOR ADDRESS: Univ. Tex. M.D. Anderson Cancer Cent., 1515 Holcombe Boulevard, Houston, TX 77030, USA**USA
JOURNAL: Proceedings of the American Association for Cancer Research Annual Meeting 36 (0): p488 1995 1995
CONFERENCE/MEETING: Eighty-sixth Annual Meeting of the American Association for Cancer Research Toronto, Ontario, Canada March 18-22, 1995; 19950318
ISSN: 0197-016X
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

1/7/19
DIALOG(R)File 5:Biosis Previews(R)

0008371923 BIOSIS NO.: 199294073764
MONOCLONAL ANTIBODIES TO %%%GELONIN%%% PRODUCTION AND CHARACTERIZATION
AUTHOR: ZIMMERMANN J (Reprint); TROMMER W E
AUTHOR ADDRESS: FB CHEMIE DER UNIV KAISERSLAUTERN, POSTFACH 3049, 6750

KAISERSLAUTERN, GER**GERMANY
JOURNAL: Hybridoma 10 (1): p65-76 1991
ISSN: 0272-457X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: BALB/c mice were immunized with %%%gelonin%%%, a 30kD glycoprotein (type 1 RIP) from the seeds of Gelonium multiflorum. By polyethylene glycol-induced %%%fusion%%% of isolated spleen cells with the myeloma cell line NS-1, three different hybridomas were obtained. Two of them were found to secrete antibodies of the IgG1 subclass, whereas the third cell line produced antibodies of the IgM type. The IgG1-secreting cell lines were adapted to serum-free medium conditions, and the antibodies were isolated from the culture supernatant. The isolated antibodies recognize independent epitopes on the %%%gelonin%%% molecule. The toxicity of %%%gelonin%%% in reticulocyte lysates was not affected when the protein was incubated with the antibodies. The IgG1s exhibit average affinity constants of about 109 M-1 and 1010 M-1, respectively, as determined by a solid-phase EIA using the avidin-biotin system.

1/7/20
DIALOG(R)File 5:Biosis Previews(R)

0005935501 BIOSIS NO.: 198835032606
TARGETED DRUG DELIVERY VIA PROTEIN-MEDIATED MEMBRANE %%%FUSION%%% SPECIFIC KILLING OF HIV-INFECTED HUMAN LYMPHOCYTES
AUTHOR: NICOLAU C (Reprint); IHLER G M; MELNICK J L; NOONAN C; GEORGE S K; TOSI F; ARVINTE T; CUDD A
AUTHOR ADDRESS: BIOPHOR CORP, COLLEGE STATION, TEX 77843, USA**USA
JOURNAL: Journal of Cellular Biochemistry Supplement (12 PART B): p254 1988
CONFERENCE/MEETING: SYMPOSIUM ON LIPOSOMES IN THE THERAPY OF INFECTIOUS DISEASES AND CANCER HELD AT THE 17TH ANNUAL MEETINGS OF THE UCLA (UNIVERSITY OF CALIFORNIA-LOS ANGELES) SYMPOSIA ON MOLECULAR AND CELLULAR BIOLOGY, LAKE TAHOE, CALIFORNIA, USA, FEBRUARY 16-20, 1988. J CELL BIOCHEM SUPPL.
ISSN: 0733-1959
DOCUMENT TYPE: Meeting
RECORD TYPE: Citation
LANGUAGE: ENGLISH

? t s2/7/1-9

2/7/1
DIALOG(R)File 5:Biosis Previews(R)

0013170735 BIOSIS NO.: 200100342574
Substrate-structure dependence of ribotoxins on cleaving RNA in C. camphora ribosome
AUTHOR: Zhang Ai-Hua; Tang Shuang; Liu Wang-yi (Reprint)
AUTHOR ADDRESS: Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences, 320 Yue-Yang Road, Shanghai, 200031, China**China
JOURNAL: Journal of Natural Toxins 10 (2): p119-125 May, 2001 2001
MEDIUM: print
ISSN: 1058-8108
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Cinnamomin, a type II ribosome-inactivating protein (RIP) isolated from the seeds of Camphora tree (Cinnamomum camphora), could not inactivate its own (autologous) ribosome. Among five RIPs (Cinnamomin A-chain, ricin A-chain, trichosanthin, %%%gelonin%%%, and soporin-S6) tested, only saporin-S6 could cleave the N-glycosidic bond of RNA in C. camphora ribosome to release a specific RNA %%%fragment%%% (R-

%%%fragment%%%) after treatment with aniline, which was shorter than that from rat liver ribosome. The amount of saporin-S6 to inactivate C. camphora ribosome was about 1000 times higher than that required for rat liver ribosome. Extra-ribosomal factors (S-100) in the post-ribosomal supernatant could not promote RNA N-glycosidase activity of cinnamomin and %%%gelonin%%% to C. camphora ribosome. These results indicated that there were some changes in the microenvironments of Sarcin/Ricin domain of C. camphora ribosome that abolished the recognition and catalysis of many RIPs. In addition, the length of C. camphora 5.8S ribosomal RNA was found to be longer than that of rat 5.8S ribosomal RNA.

2/7/2
DIALOG(R)File 5:Biosis Previews(R)

0012909543 BIOSIS NO.: 200100081382
Polynucleotide:adenosine glycosidase is the sole activity of ribosome-inactivating proteins on DNA
AUTHOR: Barbieri Luigi (Reprint); Valbonesi Paola; Righi Federica; Zuccheri Giampaolo; Monti Federica; Gorini Paola; Samori Bruno; Stirpe Fiorenzo
AUTHOR ADDRESS: Department of Experimental Pathology, University of Bologna, Via San Giacomo 14, I-40126, Bologna, Italy**Italy
JOURNAL: Journal of Biochemistry (Tokyo) 128 (5): p883-889 Nov., 2000 2000
MEDIUM: print
ISSN: 0021-924X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Polynucleotide: adenosine glycosidases (PNAG) are a class of plant and bacterial enzymes commonly known as ribosome-inactivating proteins (RIP). They are presently classified as rRNA N-glycosidases in the enzyme nomenclature (EC 3.2.2.22). Several activities on nucleic acids, other than depurination, have been attributed to PNAG: in particular modifications induced in circular plasmids, including linearisation and topological changes, and cleavage of guanidinic residues. Here we describe a chromatographic procedure to obtain nuclease-free PNAG by dye-chromatography onto Procion Red derivatized Sepharose(R). Highly purified enzymes depurinate extensively pBR322 circular, supercoiled DNA at neutral pH and exhibit neither DNase nor DNA glycolyase activities, do not cause topological changes, and adenine is the only base released from DNA and rRNA, even at very high enzyme concentrations. A scanning force microscopy (SFM) study of pBR322 treated with saporin-S6 confirmed that (i) this PNAG binds extensively to the plasmid, (ii) the distribution of the bound saporin-S6 molecules along the DNA chain is markedly variable, (iii) plasmids already digested with saporin-S6 do not appear %%%fragmented%%% or topologically modified. The observations here described demonstrate that polynucleotide:adenosine glycosidase is the sole enzymatic activity of the four ribosome-inactivating proteins %%%gelonin%%%, momordin I, pokeweed antiviral protein from seeds and saporin-S6. These proteins belong to different families, suggesting that the findings here described may be generalized to all PNAG.

2/7/3
DIALOG(R)File 5:Biosis Previews(R)

0009848131 BIOSIS NO.: 199598315964
Delivery of the ribosome-inactivating protein, %%%gelonin%%%, to lymphoma cells via CD22 and CD38 using bispecific antibodies
AUTHOR: French R R; Penney C A; Browning A C; Stirpe F; George A J T; Glennie M J (Reprint)
AUTHOR ADDRESS: Lymphoma Res. Unit, Tanovus Lab., General Hosp., Southampton SO16 6YD, UK**UK
JOURNAL: British Journal of Cancer 71 (5): p986-994 1995 1995
ISSN: 0007-0920
DOCUMENT TYPE: Article
RECORD TYPE: Abstract

LANGUAGE: English

ABSTRACT: It is well established that bispecific antibodies (BsAbs) can be used effectively in targeting the ribosome-inactivating protein (RIP), saporin, against neoplastic B cells. We have now extended this delivery system for use with %%%gelonin%%%. By measuring antigen-binding characteristics and epitope mapping a panel of anti-%%%gelonin%%% MAbs using the IAsys resonant mirror biosensor, we were able to rapidly select the most suitable for making BaAbs. The Fab' %%%fragments%%% from these MAbs were chemically conjugated with Fab' from either anti-CD22 or anti-CD38. Cytotoxicity assays showed that BsAbs were highly efficient at delivering %%%gelonin%%% to cultured Daudi cells and achieved levels of toxicity which correlated closely with the affinity of the BsAbs. Using pairs of anti-CD22 BsAbs we were able to generate bivalent BsAb-%%%gelonin%%% complexes which achieved IC-50 values of 2 times 10-11 M %%%gelonin%%%, a potency which is equivalent to that reached by saporin in this targeting system. However, because %%%gelonin%%% is 5-10 times less toxic than saporin, the therapeutic ratio for %%%gelonin%%% is superior, making it potentially a more useful agent for human treatment. Cytotoxicity assays and kinetic analysis showed that targeting %%%gelonin%%% via CD38 was 2-5 times less effective than delivery through CD22. However, with a pair of BsAbs designed to co-target %%%gelonin%%% via CD22 and CD38, the cytotoxicity achieved equalled that obtained with a pair of anti-CD22 BsAbs (IC-50 = 1 times 10-11 M). This important result suggests that the anti-CD38 helps bind the %%%gelonin%%% to the cell and is then 'dragged' or 'piggy-backed' into the cell by the anti-CD22 BsAb. The implication of these findings for cancer therapy is discussed.

2/7/4
DIALOG(R)File 5:Biosis Previews(R)

0009493623 BIOSIS NO.: 199497514908
Selective antitumor effect of thioether-linked immunotoxins composed of %%%gelonin%%% and monoclonal antibody to alpha-fetoprotein or its F(ab')-2 %%%fragment%%%
AUTHOR: Masuda Kazuyoshi (Reprint); Takahashi Koji; Hirano Koichiro; Takagishi Yasushi
AUTHOR ADDRESS: Shionogi Res. Lab., Shionogi and Co., Ltd., Fukushima-ku, Osaka 553, Japan**Japan
JOURNAL: Tumor Biology 15 (3): p175-183 1994 1994
ISSN: 1010-4283
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Two thioether-linked conjugates composed of monoclonal antibody (MoAb) to alpha-fetoprotein (AFP), 80G or its F(ab')-2 %%%fragment%%%, and a type 1 ribosome-inactivating protein (RIP), %%%gelonin%%%, were prepared as potent immunotoxins (80G-CS-GL(IT) and F(ab')-2-CS-GL(IT)). Each conjugate contained one %%%gelonin%%% per 80G or its F(ab')-2 %%%fragment%%%. The binding activity of these conjugates was as high as that of intact 80G or F(ab')-2. The in vitro cytotoxic effect of F(ab')-2-CS-GL(IT) on AFP-producing HuH-7 cells was approximately 100-fold more potent than that of 80G-CS-GL(IT). Also, F(ab')-2-CS-GL(IT) showed slight cytotoxicity against non-AFP-producing HuH-13 cells, while 80G-CS-GL(IT) did not. On the other hand, both conjugates had similar selective antitumor activity against HuH-7N cells in nude mice, possibly due to their similar distribution in the tumor. The results suggest that our MoAb 80G is a suitable carrier for delivering type 1 RIP such as %%%gelonin%%% into AFP-producing hepatoma cells and that its F(ab')-2 %%%fragmentation%%% does not enhance targeting efficiency.

2/7/5
DIALOG(R)File 5:Biosis Previews(R)

0009432369 BIOSIS NO.: 199497453654

Characterization of the increased cytotoxicity of %%%gelonin%%% anti-T cell immunoconjugates compared with ricin A chain immunoconjugates
AUTHOR: Fishwild Dianne M (Reprint); Wu H-W; Carroll S F; Bernhard S L
AUTHOR ADDRESS: GenPharm Int., 297 North Bernardo Ave., Mountain View, CA 94043, USA**USA
JOURNAL: Clinical and Experimental Immunology 97 (1): p10-18 1994 1994
ISSN: 0009-9104
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Ribosomal inactivating proteins such as %%%gelonin%%% (Gel) and ricin A chain (RTA) conjugated to MoAbs bind to specific target cells, and upon internalization inhibit protein synthesis, ultimately resulting in cell death. We report here that Gel anti-T cell MoAb conjugates are more cytotoxic than RTA conjugates when tested against human peripheral blood mononuclear cells (PBMC). This increased cytotoxicity is observed whether Gel is conjugated to the anti-T cell MoAb or to an anti-mouse immunoglobulin Fab' %%%fragment%%% which then binds to the murine anti-human T cell MoAb. Gel conjugates are not only effective at lower concentrations, but also produce a greater extent of inhibition of cellular proliferation. Moreover, a 10 min exposure to a Gel conjugate is as effective as a 90 h exposure to an RTA conjugate. When part of anti-T cell F(ab')-2 or Fab' conjugates, Gel affects the early steps in cellular intoxication more than RTA; Gel conjugates bind more avidly and accelerate the modulation of antigen. In contrast, when part of whole IgG conjugates, Gel does not affect the binding to or modulation of surface antigen compared with RTA, while it does increase conjugate cytotoxicity. These observations suggest that Gel may be delivered more efficiently into the cytosol than RTA. A divergent intracellular pathway for Gel is also supported by the inability of chemical potentiators, which strongly enhance RTA potency, to affect Gel potency. These properties of Gel might also be advantageous for targeted immunoconjugates made with other MoAbs or receptor-binding molecules.

2/7/6
DIALOG(R)File 5:Biosis Previews(R)

0009028729 BIOSIS NO.: 199497050014
Efficacy of anti-CD5 F(AB')-2 and FAB' immunoconjugates in human peripheral blood lymphocyte-reconstituted severe combined immunodeficient mice
AUTHOR: Kohn Fred R (Reprint); Fishwild Dianne M; Bernhard Susan L; Better Marc; Kung Ada H C
AUTHOR ADDRESS: Dep. Pharmacology, XOMA Corp., 2910 Seventh Street, Berkeley, CA 94710, USA**USA
JOURNAL: International Journal of Immunopharmacology 15 (8): p871-878 1993 1993
ISSN: 0192-0561
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A human peripheral blood lymphocyte-reconstituted severe combined immunodeficient (hu-PBL-SCID) mouse model was used to compare in vivo efficacy of immunoconjugates directed against the CD5 antigen present on human T-cells. Four anti-CD5 immunoconjugates were tested, composed of chimeric human-mouse (cH65) F(ab'2)-2 or Fab' %%%fragments%%% chemically linked to recombinant %%%gelonin%%% (rGEL) or the 30,000 M-r glycoform of ricin A chain (RTA-30). Immunoconjugate treatment was initiated apprx 3 weeks after PBL transplantation and consisted of five consecutive daily bolus i.v. injections. Efficacy was subsequently assessed by quantitation of human T-cells in spleens, blood and peritoneal lavage fluid using 3-color flow cytometry. cH65 F(ab')-2- and cH65 Fab'-rGEL conjugates were essentially equally effective at depleting human T-cells from SCID mouse tissues, suggesting that bivalent binding is not required for efficacy when rGEL is the cytotoxic moiety. Treatment with unconjugated F(ab')-2, unconjugated Fab' or a Fab-rGEL immunoconjugate of irrelevant binding specificity did not result in a significant depletion of T-cells, demonstrating that the cytotoxic moiety and a relevant human T-cell

binding moiety are both required for efficacy. In contrast to the results observed with the rGEL conjugates, cH65 Fab'-RTA-30 was not as effective as cH65 F(ab')-2-RTA-30 in depleting human T-cells from SCID mouse tissues. This paralleled in vitro findings in a human PBMC cytotoxicity assay, which demonstrated that cH65 Fab'-RTA-30 was 17-fold less potent than cH65 F(ab')-2-RTA-30 and apprx 50-fold less potent than the rGEL conjugates. These results indicate that the hu-PBL-SCID mouse model can be used to evaluate potential differences in efficacy of cytotoxic agents direct against human T-cells and implicate anti-CD5 conjugates containing rGEL as potentially potent therapeutic agents.

2/7/7
DIALOG(R)File 5:Biosis Previews(R)

0009020445 BIOSIS NO.: 199497041730
Structural characterization of %%%gelonin%%%: Evidence for separate antigenic and cytotoxic domains
AUTHOR: Sairam M R; Srinivasa B R; Marcil Josee
AUTHOR ADDRESS: Reprod. Res. Lab., Clin. Res. Inst. Montreal, 110 Pine Ave. West, Montreal, PQ, CAN H2W 1R7, Canada**Canada
JOURNAL: Biochemistry and Molecular Biology International 31 (3): p575-581 1993 1993
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The N-terminal sequence of the three isoforms of %%%gelonin%%% is identical. Cyanogen bromide cleavage of %%%gelonin%%% produced %%%fragments%%% of Mr 17,000, 13,000, 11,000 and 7,000. The apparent Mr 17,000 component was identified as the N-terminal %%%fragment%%% and represents the major antigenic domain of the protein as it reacted with antibody to the native protein but this %%%fragment%%% did not inhibit protein synthesis in the in vitro translation assay. Our data may suggest possibilities for separation of antigenic and catalytic domains of this ribosome inactivating protein.

2/7/8
DIALOG(R)File 5:Biosis Previews(R)

0008725858 BIOSIS NO.: 199395028124
A comparison of anti-lymphocyte immunotoxins containing different ribosome-inactivating proteins and antibodies
AUTHOR: Bolognesi A; Tazzari P L; Tassi C; Gromo G; Gobbi M; Stirpe Fiorenzo (Reprint)
AUTHOR ADDRESS: Dip. Patologia Sperimentale, Via S. Giacomo 14, I-40126 Bologna, Italy**Italy
JOURNAL: Clinical and Experimental Immunology 89 (3): p341-346 1992
ISSN: 0009-9104
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Immunotoxins were prepared with several single-chain ribosome-inactivating proteins (RIPs type 1) and with the A-chain of ricin linked to the F(ab')-2 %%%fragment%%% of sheep anti-mouse IgG. The cytotoxic activity of these conjugates was tested on human lymphocytes pretreated with an anti-CD3 murine moAb. The immunotoxins inhibited DNA synthesis in phytohaemagglutinin (PHA)-stimulated lymphocytes with IC-50s (concentrations causing 50% inhibition) ranging from 8.9 times 10-13 to 5.7 times 10-11 M (immunotoxins containing dianthin 32, saporin, pokeweed antiviral protein from seeds (PAP-S), bryodin, momordin, momorcochin, and trichokirin), 1 times 10-8 M (immunotoxin containing %%%gelonin%%%) and 5 times 10-9 M (immunotoxin containing ricin A-chain). The immunotoxin containing saporin linked to the anti-mouse IgG F(ab')-2 %%%fragment%%% was also highly toxic to human lymphocytes pretreated with anti-CD2, -CD3, -CD5 and -CD45 MoAbs, with IC-50s ltoreq 10-11 M. Immunotoxins were prepared also with saporin linked to MoAbs against various CD antigens.

The immunotoxin prepared with the anti-CD3 antibody had the highest specific cytotoxicity to human lymphocytes.

2/7/9
DIALOG(R)File 5:Biosis Previews(R)

0006127511 BIOSIS NO.: 198885096402
THE SITE OF ACTION OF SIX DIFFERENT RIBOSOME-INACTIVATING PROTEINS FROM PLANTS ON EUKARYOTIC RIBOSOMES THE RNA N GLYCOSIDASE ACTIVITY OF THE PROTEINS
AUTHOR: ENDO Y (Reprint); TSURUGI K; LAMBERT J M
AUTHOR ADDRESS: DANA-FABER CANCER INST, DIV TUMOR IMMUNOL, 44 BINNEY ST, BOSTON, MASS 02115, USA**USA
JOURNAL: Biochemical and Biophysical Research Communications 150 (3): p 1032-1036 1988
ISSN: 0006-291X
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: ENGLISH

ABSTRACT: The site of action of six different ribosome-inactivating proteins from plants on eukaryotic ribosomes was studied. Treatment of ribosomes with any one of these proteins caused the 28S rRNA extracted from the inactivated ribosomes to become sensitive to treatment with aniline. A %%%fragment%%% containing about 450 nucleotides was released from the 28S rRNA. Further analysis of the nucleotide sequences of the 450-nucleotide %%%fragments%%% revealed that the aniline-sensitive phosphodiester bond was between A-4324 and G-4325 of the 28S rRNA. These results indicate that all six ribosome-inactivating proteins damage eukaryotic ribosomes by cleaving the N-glycosidic bond at A-4324 of the 28S rRNA of the ribosome, as does ricin A-chain.

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8/7/1
DIALOG(R)File 5:Biosis Previews(R)

0014845706 BIOSIS NO.: 200400226463
Retinoic acid-induced CD38 antigen as a target for immunotoxin-mediated killing of leukemia cells.
AUTHOR: Mehta Kapil (Reprint); Ocanas Larry; Malavasi Fabio; Marks John W; %%%Rosenblum Michael G%%%
AUTHOR ADDRESS: Department of Bioimmunotherapy, University of Texas MD Anderson Cancer Center, 1515 Holcombe Boulevard, Unit 422, Houston, TX, 77030, USA**USA
AUTHOR E-MAIL ADDRESS: kmehta@mdanderson.org
JOURNAL: Molecular Cancer Therapeutics 3 (3): p345-352 March 2004 2004
MEDIUM: print
ISSN: 1535-7163 _(ISSN print)
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A major obstacle in the successful delivery of antibody-based therapeutics to tumor cells is the heterogeneity of target antigen expression. We reported previously that retinoic acid (RA) is a potent and selective inducer of the cell-surface antigen CD38 in myeloid leukemia cells. The purpose of this study was to determine whether the RA-induced CD38 antigen could be a target for an anti-CD38-based immunotoxin to induce selective killing of leukemia cells. The combination of RA and the anti-CD38 %%%gelonin%%% immunotoxin induced a synergistic killing of leukemia cells. Thus, coculture of myeloid leukemia cells and cell lines with as little as 1 nm RA in the presence of immunotoxin induced substantial killing (>90%) of leukemia cell clones. More importantly, the blasts of myeloid leukemia patients, irrespective of their morphological and phenotypic features, also responded to the RA and immunotoxin combination when cultured ex vivo. A similar synergistic effect between RA and immunotoxin was observed

against a multidrug-resistant variant subline of HL-60 cells. However, another variant of HL-60 cells, HL-60R, in which the retinoid receptor function has been abrogated by a trans-dominant-negative mutation, exhibited complete resistance to the immunotoxin-induced killing effect in the presence or absence of RA. Our results suggest that RA combined with anti-CD38-based therapeutic agent may offer exciting opportunities for the treatment of myeloid leukemias despite their multiplicity of genetic and clinical varieties.

8/7/2
DIALOG(R)File 5:Biosis Previews(R)

0014492556 BIOSIS NO.: 200300451235
In vitro and in vivo anti-angiogenic effects of VEGF121/rGel fusion toxin.
AUTHOR: Mohamedali Khalid A (Reprint); Kedar Daniel; Ran Sophia; Garcia Bobby; Dinney Colin P; Thorpe Philip; %%%Rosenblum Michael G%%%
AUTHOR ADDRESS: UT M. D. Anderson Cancer Center, Houston, TX, USA**USA
JOURNAL: Proceedings of the American Association for Cancer Research Annual Meeting 44 p7 July 2003 2003
MEDIUM: print
CONFERENCE/MEETING: 94th Annual Meeting of the American Association for Cancer Research Washington, DC, USA July 11-14, 2003; 20030711
ISSN: 0197-016X
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

8/7/3
DIALOG(R)File 5:Biosis Previews(R)

0014492534 BIOSIS NO.: 200300451213
VEGF121 %%%gelonin%%% fusion protein inhibits breast cancer metastasis in nude mice.
AUTHOR: Ran Sophia (Reprint); Mohamedali Khalid; Thorpe Philip E; %%%Rosenblum Michael G%%%
AUTHOR ADDRESS: University of Texas at Dallas, Dallas, TX, USA**USA
JOURNAL: Proceedings of the American Association for Cancer Research Annual Meeting 44 p1 July 2003 2003
MEDIUM: print
CONFERENCE/MEETING: 94th Annual Meeting of the American Association for Cancer Research Washington, DC, USA July 11-14, 2003; 20030711
ISSN: 0197-016X
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

8/7/4
DIALOG(R)File 5:Biosis Previews(R)

0014421889 BIOSIS NO.: 200300379166
Design, expression, purification, and characterization, in vitro and in vivo, of an antimelanoma single-chain Fv antibody fused to the toxin %%%gelonin%%%.
AUTHOR: %%%Rosenblum Michael G%%% (Reprint); %%%Cheung Lawrence H%%%; Liu Yuying; Marks John W
AUTHOR ADDRESS: Section of Immunopharmacology and Targeted Therapy, Department of Bioimmunotherapy, University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Unit 44, Houston, TX, 77030, USA**USA
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JOURNAL: Cancer Research 63 (14): p3995-4002 July 15, 2003 2003
MEDIUM: print
ISSN: 0008-5472 _(ISSN print)
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: We constructed a single-chain anti-gp240 antibody (designated MEL sFv) and fused this to the recombinant toxin %%%gelonin%%% (rGel). MEL sFv-rGel was produced in bacterial expression plasmid (pET-32), and the protein composition was confirmed by both DNA sequencing and Western analysis. Inhibition of cell-free protein synthesis by the fusion construct demonstrated an IC50 of 100 pM, comparable with that for native %%%gelonin%%% (104 pM). The MEL sFv-rGel fusion toxin bound to antigen-positive but not antigen-negative cells as assessed by ELISA. Internalization into A-375 target cells was demonstrable by 1 h after exposure. Against A-375 cells, MEL sFv-rGel demonstrated an IC50 of approximately 8 nM, which was 250-fold lower than that for free rGel (2000 nM). The cytotoxic effects of the construct did not involve apoptosis because terminal deoxynucleotidyl transferase-mediated nick end labeling assays of treated cells were negative. 125I-labeled MEL sFv-rGel demonstrated biphasic clearance of the construct from plasma (t1/2 alpha and t1/2 beta were 0.46 and 7.2 h, respectively). At 72 h after administration, xenograft studies showed that the tissue: blood ratio was highest for tumor followed by spleen, kidney, and liver. Groups of tumor-bearing nude mice were treated with fusion toxin at either 2 or 20 mg/kg. Compared with saline-treated controls, for which mean tumor burden increased 6-fold, the groups treated with the high and low doses of fusion construct showed no increase or only a 2-fold increase, respectively. These studies suggest that this recombinant fusion construct has potent cytotoxic activity both in vitro and in vivo and is an excellent candidate for clinical development.

8/7/5
DIALOG(R)File 5:Biosis Previews(R)

0014116218 BIOSIS NO.: 200300074937
Immunotoxin resistance in multidrug resistant cells.
AUTHOR: McGrath Melissa S; %%%Rosenblum Michael G%%%; Philips Mark R; Scheinberg David A (Reprint
AUTHOR ADDRESS: Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, NY, 10021, USA**USA
AUTHOR E-MAIL ADDRESS: d-scheinberg@ski.mskcc.org
JOURNAL: Cancer Research 63 (1): p72-79 January 1, 2003 2003
MEDIUM: print
ISSN: 0008-5472 _(ISSN print)
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Multidrug resistance (MDR) can be mediated, in part, by overexpression of P-glycoprotein (P-gp) and is characterized by broad resistance to several structurally, chemically, and pharmacologically distinct chemotherapeutic compounds. It has been hypothesized that immunological approaches to cytolysis may be used to overcome drug resistance. RV+ is a P-gp-expressing variant of the human myeloid leukemic cell line HL60 that displays a typical MDR phenotype. MDR RV+ cells displayed relative resistance to the immunotoxin (IT) HuM195-%%%gelonin%%% and to free rGelonin. K562 leukemia cells retrovirally infected to overexpress P-gp are also resistant to HuM195-%%%gelonin%%%. In addition, a monoclonal antibody capable of inhibiting the function of P-gp was able to partially reverse resistance to the IT. These data indicated that the expression of P-gp may contribute to IT resistance in RV+. Resistance to the IT was not mediated through decreased binding to cells, nor reduced internalization into the cell because the IT displayed similar kinetics of binding and internalization for both the parental HL60 and MDR RV+ cell lines. Comparison of the cytotoxicity of other ribosome-inactivating toxins indicated that RV+ cells were not universally resistant to toxins: RV+ cells were sensitive to the actions of ricin A chain, which acts on precisely the same RNase target as %%%gelonin%%%. Sensitivity of the MDR RV+ cells to the protein synthesis inhibitor cycloheximide, saponin, and Pseudomonas exotoxin A additionally confirmed that the resistance was not mediated through the ribosome and that pathways downstream from the inactivation of protein synthesis leading to cell death were not substantially perturbed in the MDR cells.

Resistance could be partially abrogated by bafilomycin A, which inhibits lysosomal function. Moreover, direct visualization by confocal microscopy of the intracellular trafficking route of the IT showed that the IT accumulated preferentially in the lysosome in MDR RV+ cells but not in sensitive cells. These observations implicated the process of increased lysosomal degradation as the most likely basis for resistance. Such pathways of resistance may be important in the therapeutic applications of ITs, now becoming available for human use.

8/7/6
DIALOG(R)File 5:Biosis Previews(R)

0013850409 BIOSIS NO.: 200200443920
Targeting bladder tumor vascular endothelium with VEGF/rGel fusion toxin
AUTHOR: Kedar Daniel (Reprint); Sweeney Paul (Reprint); Huang Samuel (Reprint); Mian Badar (Reprint); %%%Rosenblum Michael G%%% (Reprint); Dinney Colin P (Reprint
AUTHOR ADDRESS: Houston, TX, USA**USA
JOURNAL: Journal of Urology 167 (4 Supplement): p120 April, 2002 2002
MEDIUM: print
CONFERENCE/MEETING: Annual Meeting of the American Urology Association, Inc. Orlando, Florida, USA May 25-30, 2002; 20020525
ISSN: 0022-5347
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

8/7/7
DIALOG(R)File 5:Biosis Previews(R)

0013825671 BIOSIS NO.: 200200419182
VEGF121-%%%gelonin%%% fusion toxin specifically localizes to tumor blood vessels and induces robust vascular damage in human tumor xenograft models
AUTHOR: Ran Sophia (Reprint); Mohamedali Khalid; %%%Cheung Lawrence%%%; Thorpe Philip E; %%%Rosenblum Michael G%%%
AUTHOR ADDRESS: Southwestern Medical Center, UT at Dallas, Dallas, TX, USA **USA
JOURNAL: Proceedings of the American Association for Cancer Research Annual Meeting 43 p1006 March, 2002 2002
MEDIUM: print
CONFERENCE/MEETING: 93rd Annual Meeting of the American Association for Cancer Research San Francisco, California, USA April 06-10, 2002; 20020406
ISSN: 0197-016X
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

8/7/8
DIALOG(R)File 5:Biosis Previews(R)

0013808858 BIOSIS NO.: 200200402369
In vitro and in vivo studies of a VEGF121/rGelonin chimeric fusion toxin targeting the neovasculature of solid tumors
AUTHOR: Veenendaal Liesbeth M; Jin Hangqing; Ran Sophia; %%%Cheung%%% %%% Lawrence%%%; Navone Nora; Marks John W; Waltenberger Johannes; Thorpe Philip; %%%Rosenblum Michael G%%% (Reprint
AUTHOR ADDRESS: Immunopharmacology and Targeted Therapy Section, Department of Bioimmunotherapy, M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Box 44, Houston, TX, 77030-4009, USA**USA
JOURNAL: Proceedings of the National Academy of Sciences of the United States of America 99 (12): p7866-7871 June 11, 2002 2002
MEDIUM: print
ISSN: 0027-8424

DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Vascular endothelial growth factor (VEGF) plays a key role in the growth and metastasis of solid tumors. We generated a fusion protein containing VEGF121 linked by a flexible G4S tether to the toxin %%%gelonin%%% (rGel) and expressed this as a soluble protein in bacteria. Purified VEGF121/rGel migrated as an 84-kDa homodimer under non-reducing conditions. VEGF121/rGel bound to purified, immobilized Flk-1, and the binding was competed by VEGF121. Both VEGF121/rGel and VEGF121 stimulated cellular kinase insert domain receptor (KDR) phosphorylation. The VEGF121/rGel fusion construct was highly cytotoxic to endothelial cells overexpressing the KDR/Flk-1 receptor. The IC50 of the construct on dividing endothelial cells expressing 105 or more KDR/Flk-1 receptors per cell was 0.5-1 nM, as compared with 300 nM for rGel itself. Dividing endothelial cells overexpressing KDR were approximately 60-fold more sensitive to VEGF121/rGel than were nondividing cells. Endothelial cells overexpressing FLT-1 were not sensitive to the fusion protein. Human melanoma (A-375) or human prostate (PC-3) xenografts treated with the fusion construct demonstrated a reduction in tumor volume to 16% of untreated controls. The fusion construct localized selectively to PC-3 tumor vessels and caused thrombotic damage to tumor vessels with extravasation of red blood cells into the tumor bed. These studies demonstrate the successful use of VEGF121/rGel fusion construct for the targeted destruction of tumor vasculature in vivo.

8/7/9
DIALOG(R)File 5:Biosis Previews(R)

0013444812 BIOSIS NO.: 200200038323
In vitro and in vivo studies of VEGF/rGel fusion toxin targeting tumor vascular endothelium
AUTHOR: Jin Hangqing (Reprint); Ran Sophia; Thorpe Philip; %%%Rosenblum%%% %%% Michael G%%%
AUTHOR ADDRESS: University of Texas, MD Anderson Cancer Center, Houston, TX, USA**USA
JOURNAL: Proceedings of the American Association for Cancer Research Annual Meeting 42 p822 March, 2001 2001
MEDIUM: print
CONFERENCE/MEETING: 92nd Annual Meeting of the American Association for Cancer Research New Orleans, LA, USA March 24-28, 2001; 20010324
ISSN: 0197-016X
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

8/7/10
DIALOG(R)File 5:Biosis Previews(R)

0012520583 BIOSIS NO.: 200000238896
In vitro studies of a recombinant, single-chain immunotoxin recognizing the HER2/neu proto-oncogene and containing the toxin %%%gelonin%%%
AUTHOR: Marks John W (Reprint); %%%Cheung Lawrence H%%% (Reprint); %%%Rosenblum Michael G%%% (Reprint
AUTHOR ADDRESS: M D Anderson Cancer Ctr, Houston, TX, USA**USA
JOURNAL: Proceedings of the American Association for Cancer Research Annual Meeting (41): p529-530 March, 2000 2000
MEDIUM: print
CONFERENCE/MEETING: 91st Annual Meeting of the American Association for Cancer Research. San Francisco, California, USA April 01-05, 2000; 20000401
ISSN: 0197-016X
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8/7/11
DIALOG(R)File 5:Biosis Previews(R)

0012520259 BIOSIS NO.: 200000238572
In vitro and in vivo studies of VEGF121/rGel fusion toxin targeting tumor neovasculature
AUTHOR: %%%Cheung Lawrence%%% (Reprint); Veenendaal Liesbeth; Marks John W; Ran Sophia; Thorpe Philip; %%%Rosenblum Michael G%%%
AUTHOR ADDRESS: M D Anderson Cancer Ctr, Houston, TX, USA**USA
JOURNAL: Proceedings of the American Association for Cancer Research Annual Meeting (41): p387 March, 2000 2000
MEDIUM: print
CONFERENCE/MEETING: 91st Annual Meeting of the American Association for Cancer Research. San Francisco, California, USA April 01-05, 2000; 20000401
ISSN: 0197-016X
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

8/7/12
DIALOG(R)File 5:Biosis Previews(R)

0012181893 BIOSIS NO.: 199900441553
Comparative cytotoxicity and pharmacokinetics of antimelanoma immunotoxins containing either natural or recombinant %%%gelonin%%%
AUTHOR: %%%Rosenblum Michael G%%% (Reprint); Marks John W; %%%Cheung%%% %%% Lawrence H%%%
AUTHOR ADDRESS: Section of Immunopharmacology and Targeted Therapy, Department of Bioimmunotheraphy, M.D. Anderson Cancer Center, 1515 Holcombe Blvd., Houston, TX, 77030, USA**USA
JOURNAL: Cancer Chemotherapy and Pharmacology 44 (4): p343-348 Oct., 1999 1999
MEDIUM: print
ISSN: 0344-5704
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Immunotoxins are a class of targeted therapeutic agents under development by various research groups. The murine monoclonal antibody designated ZME-018 recognizes a high molecular weight glycoprotein present on most human melanoma cells and biopsy specimens and has been utilized for clinical imaging studies in patients with melanoma. The plant toxin %%%gelonin%%% is a ribosome-inactivating protein (RIP) with n-glycosidase activity similar to that of ricin A chain. In previous studies by our group, the %%%gelonin%%% toxin was sequenced, cloned and expressed in E. coli. The purified recombinant %%%gelonin%%% (RG) was found to have identical protein synthesis inhibitory activity to that of natural %%%gelonin%%% (NG). For comparative purposes, chemical conjugates of antibody ZME and either RG or NG were produced using the heterobifunctional cross-linking reagents SPDP and SMPT. The ZME-NG and ZME-RG immunotoxins were found to be 104- to 105-fold more cytotoxic to antigen-positive human melanoma cells than free toxin. NG toxin alone was cytotoxic to intact cells (IC50 = 100 nM) while RG was nontoxic to cells at doses up to 1 muM. Both ZME-NG and ZME-RG immunoconjugates were nontoxic to antigen-negative (Me-180) cells. ZME-RG immunotoxins constructed with the more stable SMPT reagent were slightly more effective in culture than conjugates made with SPDP. Tissue distribution studies in tumor-bearing nude mice demonstrated that tumor uptake of the ZME-RG immunotoxin was similar to that of the intact ZME antibody with reduced distribution to normal organs compared to an immunoconjugate produced with NG. Pharmacokinetic studies showed that the terminal-phase plasma half-life of ZME-RG was similar to that of ZME itself (42 h vs 50 h) and almost threefold higher than that of ZME-NG (11.5 h). The area under the concentration curve (Cxt) for ZME-RG was 50% lower than that for ZME due to an increased apparent volume of distribution (Vda) but was

almost tenfold higher than the Cxt for ZME-NG. These studies suggest that immunoconjugates comprising RG demonstrate identical in vitro cytotoxic effects to immunoconjugates produced with NG and immunotoxins with RG display improved in vivo pharmacodynamics and tissue distribution compared to immunotoxins containing NG.

8/7/13
DIALOG(R)File 5:Biosis Previews(R)

0010359317 BIOSIS NO.: 199698827150
Cellular resistance to the antimelanoma immunotoxin ZME-%%%gelonin%%% and strategies to target resistant cells
AUTHOR: %%%Rosenblum Michael G%%% (Reprint); %%%Cheung Lawrence%%%; Kim Se Kyu; Mujoo Kalpana; Donato Nicholas J; Murray James L
AUTHOR ADDRESS: Dep. Clin. Immunol. Biol. Ther., MD Anderson Cancer Cent., 1515 Holcombe Blvd., Box 041, Houston, TX 77030, USA**USA
JOURNAL: Cancer Immunology Immunotherapy 42 (2): p115-121 1996 1996
ISSN: 0340-7004
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The development of cellular resistance to immunotoxins has been demonstrated in a variety of models and can involve a number of mechanisms. For the present study, an immunotoxin was utilized composed of an antimelanoma antibody ZME-018 recognizing a 240-kDa surface glycoprotein (gp 240) and the plant toxin %%%gelonin%%%. Human melanoma cells (A375-M) were grown in the presence of increasing amounts of ZME-%%%gelonin%%% and a clonal variant (A-375-ZR) was developed that was 100-fold resistant to ZME-%%%gelonin%%% compared to parental cells. Scatchard analysis showed that the A375-M parental cells had 260 times 10-3 ZME-%%%gelonin%%%-binding sites/cell with relatively low affinity (5 nM). In contrast, resistant A375-ZR cells demonstrated a reduced number of low-affinity sites (160 times 10-3/cell), but showed a small number (47 times 10-3) of higher-affinity sites (0.8 nM). Internalization rates and degradation rates of 125I-labeled ZME-%%%gelonin%%% were identical in both the parental and resistant cells. A375-ZR cells were found to be more resistant to vincristine and doxorubicin than were parental cells. Both cell lines were almost equally sensitive to native %%%gelonin%%%, 5-fluorouracil (5-FU), cisplatin, melphalan. carmustine, interferon gamma (IFN-gamma) and IFN-alpha. In addition, both cell lines were equally sensitive to another %%%gelonin%%%-antibody conjugate that binds to cell-surface, GD-2 (antibody 14G-2A). However, resistant cells were twice as sensitive to the cytotoxic effects of etoposide than were parental cells. Finally, a variety of agents were tested in combination with ZME-%%%gelonin%%% against A375-ZR cells in an attempt to identify agents to augment immunotoxin cytotoxic effects against resistant cells. The agents 5-FU, cisplatin, IFN-gamma, IFN-alpha, and etoposide were the most effective in augmenting the cytotoxicity of ZME-%%%gelonin%%% against resistant cells. These studies suggest that development of resistance to one immunotoxin does not cause development of cross-resistance to other %%%gelonin%%% immunotoxins. Further, specific biological response modifiers and chemotherapeutic agents may be effective in augmenting the effectiveness of immunotoxins and specifically targeting or reducing the emergence of immunotoxin-resistant cells.

8/7/14
DIALOG(R)File 5:Biosis Previews(R)

0010311196 BIOSIS NO.: 199698779029
Potentiation of antiproliferative effects of monoclonal antibody lym-1 and immunoconjugate Lym-1-%%%gelonin%%% on human Burkitt's lymphoma cells with gamma-interferon and tumor necrosis factor
AUTHOR: O'Boyle Kevin P (Reprint); Colletti Deborah; Mazurek Constance; Wang Yuexian; Ray Subhransu K; Diamond Betty; %%%Rosenblum Michael G%%%; Epstein Alan L; Schochat Dan; Dutcher Janice P; Wiernik Peter H; Klein Robert S

AUTHOR ADDRESS: Dep. Oncol., Montefiore Med. Cent., 111 East 210th Street, The Bronx, NY 10467, USA**USA
JOURNAL: Journal of Immunotherapy with Emphasis on Tumor Immunology 18 (4): p221-230 1995 1995
ISSN: 1067-5582
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: A type I ribosome inactivating protein, %%%gelonin%%%, was linked to Lym-1, a murine monoclonal antibody reactive with a polymorphic determinant of class II HLA-DR histocompatibility leukocyte antigen (HLA) on human lymphoma cells, via a disulfide linkage using the heterobifunctional cross-linking agent, N-succinimidyl-3-(2-pyridyldithio) propionate. This immunotoxin was purified from unreacted %%%gelonin%%% and unconjugated Lym-1 by fast protein liquid chromatography using sephacryl S-300 gel filtration and blue sepharose affinity gradient separation. Binding of Lym-1-%%%gelonin%%% immunoconjugate to human Raji Burkitt's lymphoma cells was demonstrated by indirect immunofluorescence using flow cytometry. Lym-1-%%%gelonin%%% was very active in 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium salt and sulforhodamine B in vitro cytotoxicity assays against the Raji lymphoma cell line and confirmed the fact that monoclonal antibody Lym-1 internalizes into human lymphoma cells. A weaker cytostatic antiproliferative effect was also noted for unconjugated Lym-1. gamma-interferon augmented the antiproliferative effects of Lym-1-%%%gelonin%%% conjugate and unconjugated Lym-1, by having a direct cytotoxic effect on the Raji cells. Tumor necrosis factor-alpha also enhanced the antiproliferative effect of unconjugated Lym-1, but did not significantly augment the cytotoxic activity of the Lym-1-%%%gelonin%%% conjugate. These results suggest that anti-HLA class II monoclonal antibodies may be useful in constructing immunotoxins for the treatment of human lymphomas and leukemias expressing HLA class II antigens, and that unconjugated anti-HLA class II monoclonal antibodies may be therapeutically useful in conjunction with recombinant cytokines, especially gamma-interferon.

8/7/15
DIALOG(R)File 5:Biosis Previews(R)

0009941968 BIOSIS NO.: 199598409801
Pharmacokinetics, tissue distribution and in vivo antitumor effects of the antimelanoma immunotoxin ZME-%%%gelonin%%%
AUTHOR: Mujoo Kalpana (Reprint); %%%Cheung Lawrence%%%; Murray James L; %%%Rosenblum Michael G%%% (Reprint
AUTHOR ADDRESS: Dep. Clin. Immunol. Biol. Therapy, Univ. Texas, M.D. Anderson Cancer Cent., 1515 Holcombe Blvd., Box 041, Houston, TX 77030, USA**USA
JOURNAL: Cancer Immunology Immunotherapy 40 (5): p339-345 1995 1995
ISSN: 0340-7004
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Antibody ZME-018 is directed against the gp240 glycoprotein on the surface of more than 80% of human melanoma cell lines and fresh biopsy specimens. Previous studies in our laboratory described the in vitro cytotoxicity and specificity of an immunoconjugate composed of mAb ZME-018 and the plant toxin %%%gelonin%%%. The present study describes the in vivo pharmacokinetics and therapeutic effects of ZME-%%%gelonin%%% in human xenograft/nude mouse models. Pharmacokinetic studies of 125I-labeled ZME-018 and ZME-%%%gelonin%%% demonstrated a shorter terminal-phase plasma half-life of the immunoconjugate than native ZME (20.6 h compared to 41.3 h). The initial volume of distribution of the ZME-%%%gelonin%%% was also higher compared to that of ZME alone (2.85 ml compared to 1.91 ml) suggesting an enhanced distribution of the conjugate outside the vasculature. The corresponding area under the concentration/time curve for the ZME-%%%gelonin%%% conjugate was 40%

lower than that of ZME alone (80.8 compared to 139.6 mu-Ci cntdot ml-1 times min). In nude mice bearing well-developed human tumor A375 melanoma xenografts, administration of 125I-labeled ZME and ZME-%%%gelonin%%% resulted in tumor-to-blood ratios of 1.9 +- 0.5 and 1.5 +- 0.6 respectively by 72 h. Compared with ZME, ZME-%%%gelonin%%% conjugate caused an increase in the content of radiolabel in kidney, spleen and liver. Treatment of nude mice bearing well-developed (150 mm-3) s.c. A375-M xenografts with divided doses of ZME-%%%gelonin%%%, ZME, %%%gelonin%%%, or saline resulted in suppression of tumor growth in the immunotoxin group but virtually no retardation of tumor growth in the control groups. Using a murine model for a rapidly growing lethal metastatic human melanoma, treatment with ZME-%%%gelonin%%% resulted in a mean survival of 44 days, a 213% increase in mean survival time compared with the saline treatment (14.2 +- 2 day survival). Given these encouraging results, we are proceeding with further preclinical development of this immunotoxin.

8/7/16
DIALOG(R)File 5:Biosis Previews(R)

0009908512 BIOSIS NO.: 199598376345
Amino Acid Sequence Analysis, Gene Construction, Cloning, and Expression of %%%Gelonin%%%, a Toxin Derived from Gelonium multiflorum
AUTHOR: %%%Rosenblum Michael G%%% (Reprint); Kohr William A; Beattie Kenneth L; Beattie Wanda G; Marks William; Toman P David; %%%Cheung%%% %%% Lawrence%%%
AUTHOR ADDRESS: Immunopharmacol. Sect., Dep. Clin. Immunol., Univ. Texas-M.D. Anderson Cancer Cent., 1515 Holcombe Boulevard, Box 041, Houston, TX 77030, USA**USA
JOURNAL: Journal of Interferon and Cytokine Research 15 (6): p547-555 1995 1995
ISSN: 1079-9907
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The plant toxin %%%gelonin%%% is an extremely potent inhibitor of protein synthesis, similar in action to ricin. The mature protein primary sequence was obtained using conventional sequencing techniques. %%%Gelonin%%% was found to be composed of 258 amino acids and contains 21 lysine residues. This toxin shares approximately 33% sequence homology with trichosanthin and ricin A chain. A 774 hp synthetic gene encoding %%%gelonin%%% was synthesized and expressed in E. coli. Recombinant %%%gelonin%%% (apprx 28 kD) expression was monitored and demonstrated by western analysis. Purification and functional activity studies demonstrated that this protein behaves identically to that of the natural product. Recombinant %%%gelonin%%% (RG) thus joins a growing list of recombinant toxins currently available for use in the construction of recombinant immunotoxins composed of %%%gelonin%%% fused to binding domains of antibodies, growth factors, or other cytokines.

8/7/17
DIALOG(R)File 5:Biosis Previews(R)

0009733558 BIOSIS NO.: 199598201391
Cloning a single-chain analogue of the anti-melanoma monoclonal antibody ZME-018: Construction and testing of an antibody-recombinant %%%gelonin%%% fusion toxin
AUTHOR: Parakh Cushrow R; %%%Cheung Lawrence%%%; %%%Rosenblum Michael G%%%
AUTHOR ADDRESS: Univ. Tex. M. D. Anderson Cancer Cent., 1515 Holcombe Boulevard, Houston, TX 77030, USA**USA
JOURNAL: Proceedings of the American Association for Cancer Research Annual Meeting 36 (0): p488 1995 1995
CONFERENCE/MEETING: Eighty-sixth Annual Meeting of the American Association for Cancer Research Toronto, Ontario, Canada March 18-22, 1995; 19950318
ISSN: 0197-016X
DOCUMENT TYPE: Meeting; Meeting Abstract

RECORD TYPE: Citation
LANGUAGE: English

8/7/18
DIALOG(R)File 5:Biosis Previews(R)

0009733557 BIOSIS NO.: 199598201390
Construction and testing of a recombinant single-chain antibody-%%%gelonin%%% fusion toxin directed against the cell surface growth factor receptor p185-HER-2/neu
AUTHOR: Parakh Cushrown R (Reprint); %%%Cheung Lawrence%%% (Reprint); King C Richter; Sastry-Dent Lakshmi; %%%Rosenblum Michael G%%% (Reprint
AUTHOR ADDRESS: Univ. Tex. M.D. Anderson Cancer Cent., 1515 Holcombe Boulevard, Houston, TX 77030, USA**USA
JOURNAL: Proceedings of the American Association for Cancer Research Annual Meeting 36 (0): p488 1995 1995
CONFERENCE/MEETING: Eighty-sixth Annual Meeting of the American Association for Cancer Research Toronto, Ontario, Canada March 18-22, 1995; 19950318
ISSN: 0197-016X
DOCUMENT TYPE: Meeting; Meeting Abstract
RECORD TYPE: Citation
LANGUAGE: English

8/7/19
DIALOG(R)File 5:Biosis Previews(R)

0009627499 BIOSIS NO.: 199598095332
Characterization of murine, and humanized anti-CD33, %%%gelonin%%% immunotoxins reactive against myeloid leukemias
AUTHOR: McGraw Kimberly J; %%%Rosenblum Michael G%%%; %%%Cheung Lawrence%%% ; Scheinberg David A (Reprint
AUTHOR ADDRESS: Memorial Sloan-Kettering Cancer Cent., New York, NY 10021, USA**USA
JOURNAL: Cancer Immunology Immunotherapy 39 (6): p367-374 1994 1994
ISSN: 0340-7004
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: M194 antibodies recognize CD33, an antigen present on acute myeloid leukemia blasts as well as some myeloid progenitor cells, but not on the ultimate hematopoietic progenitor stem cell. Immunotoxins (IT) reactive with human myeloid leukemias were constructed by conjugating %%%gelonin%%%, a single-chain ribosome-inactivating protein, to murine and genetically engineered, humanized M195 antibodies via an N-succinimidyl-3-(2-pyridyldithio)-proprionate linkage. No losses of %%%gelonin%%% cytotoxic activity or M195 binding activity were observed after conjugation of up to two toxin molecules per antibody. Toxin conjugates displayed specific, potent toxicity for CD33+ cells. The murine and humanized IT were not toxic to CD33- cells and were 600 and 4500 times more potent, respectively, than free %%%gelonin%%% in inhibiting CD33+ HL60 cells. Treatment of HL60 cells with 1 mu-g/ml HuM195-%%%gelonin%%% resulted in more than 100 times lower colony formation; normal bone marrow mononuclear cell colony-forming units treated with HuM195-IT were reduced by a factor of 10. HL60 leukemia cells could be effectively purged from an excess of normal bone marrow cells. Exposure of target cells to IT for as little as 30 min was as effective as continuous exposure of IT for up to 6 days. However, measures of the efficacy of the immunotoxin were directly related to the length of time of observation after IT exposure and were inversely related to cell concentration. M195-%%%gelonin%%% immunoconjugates are potential candidates for therapeutic use in vivo or ex vivo bone marrow purging of myeloid leukemias.

8/7/20
DIALOG(R)File 5:Biosis Previews(R)

0008727563 BIOSIS NO.: 199395029829
A %%%gelonin%%%-containing immunotoxin directed against human breast carcinoma
AUTHOR: %%%Rosenblum Michael G%%% (Reprint); Zuckerman Joan E; Marks John W ; Rotbein Judith; Allen W Ross
AUTHOR ADDRESS: Immunopharmacol. Sect., Dep. Clin. Immunol. and Biological Therapy, Univ. Tex. M.D. Anderson Cancer Cent., 1515 Holcombe Blvd., Box 041, Houston, Tex. 77030, USA**USA
JOURNAL: Molecular Biotherapy 4 (3): p122-129 1992
ISSN: 0952-8172
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Toxins may be specifically directed to tumor cells and the toxins' potency greatly increased by covalent conjugation to monoclonal antibodies recognizing tumor-associated antigens. Antibody 15A8, an immunoglobulin G-1 (IgG-1) subclass anti-human breast carcinoma murine monoclonal antibody and %%%gelonin%%%, a plant toxin, were covalently modified with N-succimindyl 3-(2-pyridyldithio) proprionate and iminothiolane, respectively, and allowed to cross-link. 15A8-%%%gelonin%%% conjugates were purified from unreacted antibody and free %%%gelonin%%% by gel filtration and blue sepharose chromatography. Analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis showed that the final product contained two bands corresponding to antibody:%%%gelonin%%% conjugates of 1:1 (predominant) and 1:2. There were no contaminating amounts of free antibody or free toxin in the preparation. The yield of the final purified 15A8-%%%gelonin%%% conjugate was approximately 20% based on the amount of starting antibody. The protein synthesis inhibitory activity of the immunoconjugate was assessed by in vitro rabbit reticulocyte translation assay. This functional activity was normalized to that of unmodified %%%gelonin%%% for use in in vitro antiproliferative assays against antigen-negative (Hs294t human melanoma) and antigen-positive (ME-180 human cervical carcinoma) cell lines. Antigen-negative Hs294t cells incubated for 72 hours with 15A8-%%%gelonin%%% immunotoxin showed no increased cytotoxicity compared with HS294t cells exposed to free %%%gelonin%%% alone. However, the immunotoxin was preferentially toxic to antigen-positive ME-180 cells; over 5 logs greater cell kill was observed after 72 hours exposure to 15A8-%%%gelonin%%% than after the same exposure to %%%gelonin%%% alone. Various lysosomotropic agents augmented 15A8-%%%gelonin%%% cytotoxicity; the most effective potentiating agent appeared to be monensin. In addition, the chemotherapeutic agents L-phenylalanine mustard (L-PAM), 5-fluorouracil, vincristine, and bleomycin and the biological response modifiers interferon-alpha and tumor necrosis factor-alpha were shown to augment 15A8-%%%gelonin%%% cytotoxicity. Should in vivo pharmacology and therapeutic studies confirm these in vitro findings, 15A8-%%%gelonin%%% conjugate may be a potent agent for therapy of cancer in man.

8/7/21
DIALOG(R)File 5:Biosis Previews(R)

0008585155 BIOSIS NO.: 199345016135
TAb 250-%%%gelonin%%%: An immunotoxin effective at inhibiting growth of tumor cells that overexpress the c-erbB-2 protein
AUTHOR: Langton Beatrice C (Reprint); Brink Jody R (Reprint); Schraufnagel Wendy L (Reprint); Shawver Laura K (Reprint); %%%Cheung Lawrence%%%; %%%Rosenblum Michael G%%%
AUTHOR ADDRESS: Dep. Oncol., Berlex Biosci., Alameda, CA, USA**USA
JOURNAL: Journal of Cellular Biochemistry Supplement 0 (17 PART D): p178 1993
CONFERENCE/MEETING: Keystone Symposium on Discovery and Development of Therapeutic Compounds Snowmass, Colorado, USA March 20-26, 1993; 19930320
ISSN: 0733-1959
DOCUMENT TYPE: Meeting
RECORD TYPE: Citation
LANGUAGE: English

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